CHARACTERIZATION AND COLISTIN SUSCEPTIBILITY OF CARBAPENEM RESISTANT ISOLATES OF *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII* IN A TERTIARY CARE HOSPITAL

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CERTIFICATE

This is to certify that this dissertation titled **"CHARACTERIZATION AND COLISTIN SUSCEPTIBILITY OF CARBAPENEM RESISTANT ISOLATES OF *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII* IN A TERTIARY CARE HOSPITAL"** is a bonafide record of work done by **Dr.M.SOWNDARYA**, during the period of April 2016 to March 2017 under the guidance of **Prof.Dr.THASNEEM BANU.S. M.D.,** Professor of Microbiology, Institute of Microbiology , Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai - 600003, in partial fulfillment of the requirement of M.D. MICROBIOLOGY Degree Examination of The Tamilnadu Dr.M.G.R. Medical University to be held in May 2018.

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DECLARATION

I, **Dr.M.SOWNDARYA**, Post Graduate , Institute of Microbiology, Madras Medical College, solemnly declare that the dissertation titled **"CHARACTERIZATION AND COLISTIN SUSCEPTIBILITY OF CARBAPENEM RESISTANT ISOLATES OF *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII* IN A TERTIARY CARE HOSPITAL"**is the bonafide work done by me at Institute of Microbiology, Madras Medical College under the expert guidance and supervision of **Prof.Dr. THASNEEM BANU.S M.D.,** Professor, Institute of Microbiology, Madras Medical College. The dissertation is submitted to the Tamil Nadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch IV) in Microbiology.

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LIST OF ABBREVIATIONS

ACT	–	AmpC Type
AmpC	–	AmpC β-lactamases
ATCC	–	American Type Culture Collection
bla	–	β-lactamases
CAUTI	–	Catheter Associated Urinary Tract Infection
CDC	–	Centre for Disease Control and prevention
CLABSI	–	Central Line Associated Blood Stream Infection
CLSI	–	Clinical Laboratory Standard Institute
CMY	–	Cephamycin hydrolysing
CNS	–	Central Nervous System
CRAB	–	Carbapenem Resistant *Acinetobacter baumannii*
CRPA	–	Carbapenem Resistant *Pseudomonas aeruginosa*
CTX-M	–	Cefotaxime hydrolyzing enzyme
CV	–	Clavulanic acid
DHP	–	Dehydropeptidase
DNA	–	Deoxy ribonucleic acid
DPA	–	Di picolinic Acid
EDTA	–	Ethylene Diamine Tetra Acetate
ESBL	–	Extended Spectrum β-lactamases
E-test	–	Epsilometer test
FOX	–	Cefoxitin

GIM	–	German Imipenemase
GNB	–	Gram-negative bacilli
ICU	–	Intensive Care Unit
IMP	–	Imipenemase
IRMR	–	Imipenem Resistant Meropenem Resistant
IRMS	–	Imipenem Resistant Meropenem Sensitive
IS	–	Insertion Sequence
kDA	–	Kilo Dalton
KPC	–	*Klebsiella pneumoniae* carbapenemase
LPS	–	Lipopolysaccharide
MBL	–	Metallo β-lactamases
mcr	–	mobilizable colisin resistance
MDR	–	Multidrug Resistant
MDRO	–	Multidrug Resistant Organism
MHT	–	Modified Hodge Test
MIC	–	Minimal Inhibitory Concentration
MIR	–	Miriam Hospital
MRIS	–	Meropenem Resistant Imipenem Sensitive
MRSA	–	Methicillin Resistant *Staphylococcus aureus*
NDM	–	New Delhi Metallo β-lactamase
NFGNB	–	Non-fermenting Gram-negative Bacilli
NHSN	–	National Healthcare Safety Network
OF	–	Oxidative/ Fermentative

OMP	–	Outer Membrane Protein
OXA	–	Oxacillinase
PBA	–	Phenyl Boronic Acid
PBP	–	Penicillin Binding Protein
PCR	–	Polymerase Chain Reaction
PER-1	–	*Pseudomonas* Extended Resistant β-lactamase
PTZ	–	Piperacillin-Tazobactam
RND	–	Resistant Nodulation Division
rRNA	–	Ribosomal Ribonucleic Acid
SENTRY	–	Antimicrobial Surveillance Program
SHV	–	Sulfhydryl variable
SIM	–	Seoul imipenemase
SME	–	*Serratia marcescens* enzyme
SPM	–	Sao Paulo metallo-β-lactamase
SSI	–	Surgical Site Infections
TEM	–	Temoneira
VAP	–	Ventilator Associated Pneumonia
VEB	–	Vietnam extended spectrum β-lactamase
VIM	–	Verona Integron encoded Metallo β-lactamase
WHO	–	World Health Organization

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Introduction

INTRODUCTION

Antimicrobial resistance is on the rise and it is a major public health problem across the world, especially in developing countries like India.[1] The continuing emergence of resistant strains causing nosocomial infections contributes to the morbidity and mortality among hospitalized patients. Of the nosocomial pathogens, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are of greatest concern for patients admitted in intensive care units [ICU].[2]

Surgical site infections, urinary tract infections, ventilator associated pneumonia and bacteremia are serious infections caused by them especially in critically ill and immunocompromised.[3] Management of these infections is difficult, as many strains often develop intrinsic and acquired resistance to multiple classes of antimicrobial drugs.[4]

Multi-drug resistant organisms (MDRO) are those organisms which are resistant to at least one agent in at least three antimicrobial classes of Cephalosporins, β-lactam/β-lactamase inhibitors, Carbapenems, Fluoroquinolones, Aminoglycosides.[5] Various mechanisms for MDR include loss of outer membrane protein, overexpression of efflux pump, production of β-lactam hydrolyzing enzymes such as extended spectrum β-lactamases (ESBL)& AmpC β-lactamases and carbapenem hydrolyzing enzymes (metallo-β-lactamases, oxacillinase).[6]

The introduction of carbapenem antibiotics such as meropenem and imipenem into clinical practice was of great help in the treatment of serious infections caused by the ESBL and AmpC producing multidrug-resistant (MDR) bacteria.[4] However, the resistance to these drugs is also on the rise because of emergence of metallo β-lactamases (MBL) and OXA type carbapenemases, which is seen predominantly in *Acinetobacter baumannii*.

Globally, reports on the carbapenemase-producing non-fermenting Gram-negative bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are on the rise due to the increased carbapenem usage and selection pressure. In India, carbapenem resistance ranges from 10.9 - 69% in *Pseudomonas aeruginosa* and 9.1-100% in *Acinetobacter baumannii* has been reported among various patient populations in differet sample types, predominantly from respiratory specimens and pus samples.[7,8,9,10]

As the production of the carbapenem hydrolyzing enzymeis plasmid mediated, it limits the therapeutic options and is a matter of serious concern for infection control management.[11] Therefore, early identification and detection of isolates that produce these enzymes are essential to avoid therapeutic failures and nosocomial outbreaks.[3] World Health Organization (WHO) has categorized carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* as Priority 1- Critical organisms for the research and development of newer antibiotics.[12]

Colistin (Polymyxin E), was one of the earliest polymyxin antibiotics, used for the treatment of gram-negative bacterial infections; however, side effects such as nephrotoxicity, and the development of less toxic antibiotics, led to its withdrawal from general use. The appearance of multidrug resistant strains of *A.baumannii* and *P. aeruginosa* has once again led to the reconsideration of colistin for the treatment of carbapenem resistant gram-negative bacterial infections.[1,13] Susceptibility testing for colistin should be carried out prior to administration to prevent treatment failure.

There is enormous geographic variation in the prevalence of antimicrobial resistance; therefore the resistance profile of resistant strains requires enhanced monitoring, especially for selection of empirical antibiotic. Obtaining regional resistance data is important for establishing guidelines for appropriate antibiotic use, and may help to control the rate of antibiotic resistance.[13]

In this background this study aims to determine the prevalence of carbapenem resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates among various clinical samples, to characterize the prevalent resistance mechanisms phenotypically and genotypically and to evaluate the *in vitro* susceptibility of colistin against the carbapenem resistant isolates.

Aims & Objectives

AIMS AND OBJECTIVES

- To isolate and identify *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates from various samples.
- To study the antimicrobial susceptibility pattern of the isolates.
- To characterize the carbapenem resistant isolates phenotypically and genotypically.
- To study the colistin susceptibility among carbapenem resistant isolates.

Review of Literature

REVIEW OF LITERATURE

Pseudomonas aeruginosa

Pseudomonads belong to the genus gammaproteobacteria, of family *Pseudomonadaceae*. Pseudomonads are aerobic, rod-shaped, gram-negative bacteria, motile by means of polar flagella. They are not acid-fast and do not form spores. The guanine and cytosine (G+C) content of the DNA ranges from 57 to 70 mol%. The pseudomonads are non-exacting and grow normally using simple sources of carbon and nitrogen.[14] The most important pseudomonads causing human infections are *Pseudomonas aeruginosa*, members of the *Burkholderia cepacia* complex and *Burkholderia pseudomallei.*[15]

CLASSIFICATION OF MEDICALLY IMPORTANT PSEUDOMONADS[16,17]

Classification of pseudomonads is based on rRNA/DNA homology and cultural characteristics.

Table 1: Classification of medically important pseudomonads

rRNA Homology group and subgroup		Genus and species
I	Fluorescent group	*Pseudomonas aeruginosa*
	Non-fluorescent group	*P.fluorescenes*
		P.putida
		P.stutzeri
		P.mendocina
II		*Burkholderia pseudomallei*
		B.mallei
		B.cepacia
		Cupriavidus
		Delftia
		Pandoraea
		Ralstonia picketti
III		*Comamonas* species *Acidovorax* species
IV		*Brevundimonas* species
V		*Stenotrophomonas maltophilia*

Pseudomonas aeruginosa is the species most commonly associated with human diseases. There are several reasons for *Pseudomonas aeruginosa* acting as an opportunistic human pathogen:

- adaptability
- innate resistance to many antibiotics and disinfectants
- putative virulence factors
- increasing number of patients compromised by age, underlying disease or immunosuppressive therapy.[15]

MORPHOLOGY AND IDENTIFICATION

MICROSCOPY

Pseudomonas aeruginosa is a motile rod shaped bacterium with single polar flagellum measuring about 0.5X 0.8 µm, and it is gram-negative.Mucoid strains may be distinguished on direct microscopic examination by the presence of clusters of short gram-negative bacilli surrounded by dark pink staining material (alginate).It is non-sporing and non-acid fast. Fimbriae may be present which are polar and non-haemagglutinating.[6,16,18]

CULTURAL CHARACTERISTICS

P.aeruginosa is an obligate aerobe, grows readily on ordinary culture media, producing a sweet or grape like corn taco-like odour. Some strains are hemolytic. *P.aeruginosa* grows well at 37-42ºC; growth at 42ºC differentiates it from other pseudomonads in the fluorescent group (*P.fluorescens, P.putida).*[16]

COLONY MORPHOLOGY

P.aeruginosa in a solid culture mediaproduces various types of colonies.

1. Circular, smooth, translucent, homogeneous, gray white colonies with entire edges. The consistency is soft.
2. Irregular, contoured, translucent gray white colonies with “beaten copper appearance”. The consistency is soft.
3. Dry, flat, opaque, granular, gray white colonies. The consistency is almost friable.

4. Mucoid, shining, whitish or grayish-green colonies; larger than other colony types. The consistency is soft, more or less viscid.
5. Rugose, opaque, granular, gray white colonies. The surface is wrinkled, with irregular or radiant crests, and usually dry. The consistency is membranaceous.[11]

PIGMENT PRODUCTION

P.aeruginosa produces four types of water soluble (diffusible) pigments when grown on nutrient agar.

1. Pyocyanin
2. Pyoverdin (Fluorescein)
3. Pyomelanin
4. Pyorubin

PYOCYANIN

Pyocyanin is a blue, non-fluorescent, water and chloroform soluble pigment, diffuses into the surrounding medium. It is produced exclusively by *Pseudomonas aeruginosa.*It is formed best in peptone media. When pyocyanin is produced in small amounts, or when its presence is obscured by other pigments, it can be observed by shaking a few milliliters of chloroform in a broth culture or an agar slope; on standing pyocyanin will appear in chloroform once the phases are separated (Chloroform extraction).[18]

PYOVERDIN

Pyoverdin is yellow, fluorescent water soluble pigment formed only in the presence of phosphate and chloroform insoluble. It is produced by all members of the fluorescent group. Pyoverdin is best observed when the cultures are illuminated by Ultra Violet (UV) light under a dark background.

PYOMELANIN

It is a dark brown pigment produced by some strains of *P.aeruginosa*. 1% tyrosine enhances the production of pyomelanin.

PYORUBIN

Few strains of *P.aeruginosa* produce a red colour pigment, Pyorubin. Growth in 1% DL-Glutamate enhances production of pyorubin.[18]

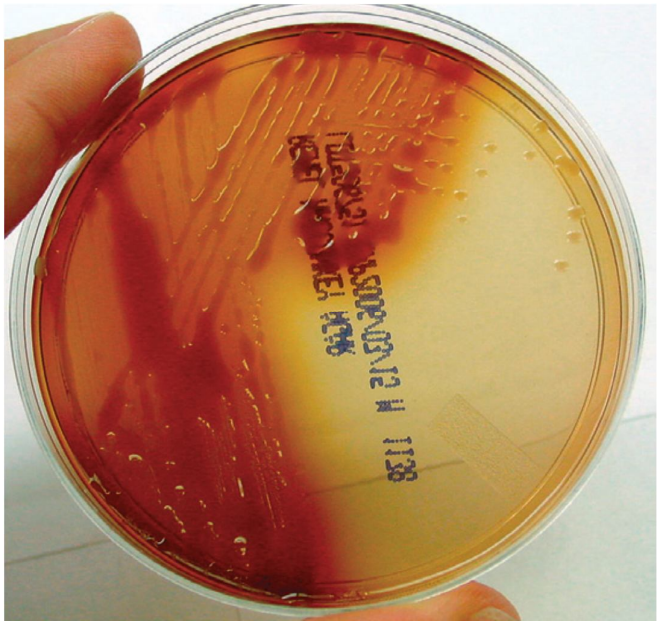


Fig:1 *P.aeruginosa* on MacConkey agar – Mucoid non-lactose fermenting colonies

Fig:2 *P.aeruginosa* colonies on blood agar with metallic sheen

BIOCHEMICAL REACTIONS

Catalase – positive, oxidase – positive, reduces nitrate to nitrite and nitrogen gas, non-fermenter – oxidatively uses glucose, maltose and mannitol variable, dihydrolyses arginine, utilizes citrate as a sole source of carbon, utilizes acetamide, liquefies gelatin, urea hydrolysis – variable, do not produce H_2S, sensitive to Polymyxin B 300U.[17]

SENSITIVITY TO PHYSICAL & CHEMICAL AGENTS

P.aeruginosa is being killed at 55ºC in one hour, but exhibits a high degree of resistance to chemical agents. It is susceptible to acids, beta glutaraldehyde, silver salts and strong phenolic disinfectants. It is resistant to common antiseptics and disinfectants such as chloroxylenol, quarternary ammonium salts, hexachlorophene and cetrimide.[18,19]

VIRULENCE FACTORS

- **Alginate** – Capsular polysaccharide permitting infecting bacteria to adhere to lung epithelial cells and forms biofilms which protects the bacteria from the host immune system and antibiotics.
- **Pili** – Surface appendages facilitating the adherence of organism to GM-1 ganglioside receptor on host epithelial cell surface.
- **Neuraminidase** – facilitates binding of pili by removing sialic acid residues from GM-1 ganglioside receptors
- **Lipopolysaccharide** – Endotoxin; causes sepsis, fever, shock, leucopenia or leucocytosis, oliguria, disseminated intravascular coagulation and metabolic abnormalities.
- **Endotoxin A**–causes tissue destruction by inhibiting protein synthesis, interrupting cell activity and macrophage response.
- **Enterotoxin** – causes diarrhoea by interrupting normal gastro-intestinal activity.
- **Exoenzyme S** – acts by inhibiting protein synthesis.
- **Phospholipase C** – inactivates opsonins, destroys cytoplasmic membrane and pulmonary surfactant.
- **Elastase** – disrupts neutrophil activity, cleaves immunoglobulin and complement components.
- **Leukocidin** – inhibits lymphocyte and neutrophil function.

- **Pyocyanin** – suppresses other bacteria, disrupts respiratory ciliary activity and causes oxidative damage to tissues, particularly oxygenated tissues such as lung.[17]

TYPING METHODS

Typing of *P.aeruginosa* is useful for epidemiological typing to establish the origin of strains causing infections, and is very important to guide treatment in environments of limited dimensions.

- Bacteriocin typing
- Serological typing

BACTERIOCIN TYPING

Bacteriocins are proteins produced by one strain of bacteria that are lethal against the cells of other strains of the same species. Pyocins are the bacteriocins produced by *P.aeruginosa*, used to classify *P. aeruginosa*. There are four categories of pyocins.

1. R type – resembling the tail of bacteriophages
2. F type - flexuous filaments
3. Low molecular weight trypsin-sensitive S type
4. Low molecular weight trypsin-resistant S type

Individual strains of *Pseudomonas aeruginosa* may produce more than one category of pyocin and also possess receptors for several different pyocins. Individual pyocins can be recognised based on their spectrum of activity against

different strains of *P.aeruginosa*. The pyocin produced by an unknown strain of *P.aeruginosa* is tested against a series of indicator strains.[14,18]

SEROLOGICAL TYPING

Serological typing is mainly used for epidemiological purpose. Nineteen group specific, heat stable O antigens and two heat labile H antigens have been recognized based on slide agglutination test.[14,18]

CLINICAL SIGNIFICANCE

P. aeruginosa causes both localized and systemic illness. Any tissue or organ system may be affected. Individuals who are at risk include those with impaired immune defenses.

1. LOCALIZED INFECTIONS

- Eye infections such as keratitis and endophthalmitis following trauma
- Ear infections causing external otitis, or swimmer's ear, and invasive and necrotizing otitis externa (malignant otitis externa) particularly in older diabetic patients
- Skin infections such as wound infection and pustular rashes
- Urinary tract infections particularly in hospitalized patients due to catheterization, instrumentation, surgery, or renal transplantation
- Respiratory tract infections causing pneumonia in individuals with chronic lung disease, congestive heart failure, or cystic fibrosis, particularly in patients who have been intubated or are on ventilators for longer period

- Gastrointestinal tract infections ranging from mild diarrheal illness in children to severe, necrotizing enterocolitis in infants and neutropenic cancer patients
- CNS infections causing meningitis and brain abscesses

Localized infections have the potential to lead to disseminated infection in immunocompromised individuals.[20]

2. SYSTEMIC INFECTIONS

Infections indicating systemic spread of the organism include bacteremia (most common in immunocompromised patients), secondary pneumonia, bone and joint infections (in intravenous drug users and patients with urinary tract or pelvic infections), endocarditis (in intravenous drug users and patients with prosthetic heart valves), central nervous system CNS (when the meninges are breached), and skin and soft tissue infections.[20]

P. aeruginosa is feared because it can cause severe nosocomial infections, especially in immunocompromised hosts. Often it is resistant to many antibiotics limiting the treatment option.

MECHANISM OF ANTIMICROBIAL RESISTANCE

Pseudomonas aeruginosa has two main mechanisms of resistance

- Intrinsic resistance
- Acquired resistance

INTRINSIC RESISTANCE

Intrinsic resistance is defined as the innate or inherent antimicrobial resistance, which is reflected in wild type antimicrobial patterns of all or almost all representatives of a species. Intrinsic resistance is so common that the susceptibility testing is unnecessary. It is mainly due to over expression of efflux pumps (*mexAB, mexCD, mexEF and mexXY*), inducible chromosomal hyper *ampC* production and loss of porins (*oprD*).[6,21]

Pseudomonas aeruginosa is intrinsically resistant to amoxicillin, ampicillin, ampicillin-sulbactam, amoxicillin-clavulanate, cefotaxime, ceftriaxone, ertapenem, trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol.[21]

EXTRINSIC RESISTANCE

This is the acquired resistance to an antimicrobial agent due to the acquisition of genes coding for resistance. Acquired resistance is due to

- Over use and misuse of an antibiotic is the most common cause.
- Evolution of strains is a natural phenomenon, which can occur among bacteria when an antibiotic is over used.
- Use of particular antibiotic poses selective pressure in a population of bacteria which promotes resistant bacteria to thrive and the susceptible bacteria to die off.

This is of importance as the resistant strains which can tolerate harsh environments, then spread in the environment and transfer the genes coding for resistance to other unrelated bacteria.[22]

Extrinsic mechanisms include acquisition of resistance genes such as extended spectrum beta-lactamases (bla_{SHV}, bla_{TEM}, bla_{VEB}, bla_{PER}) and carbapenemases (bla_{VIM}, bla_{NDM}, bla_{IMP}, $bla_{KPC,}$ bla_{SPM}).[1]

Table 2: CLASSIFICATION OF BETA-LACTAMASES[6,17]

Bush-Jacoby classification (2010)	Ambler Molecular class	Enzymes	Active site	Enzyme inhibitors	Found in
Group 1 Cephalosporinase	C	AmpC, ACT-1, CMY-2, FOX-1, MIR-1	Serine	PBA, DPA, Cloxacillin	*Enterobacteriaceae, Acinetobacter spp*.
Group 1e Cephalosporinase	C	GC1, CMY-37	Serine	Not inhibited by CV or PTZ	*Enterobacteriaceae*
Group 2a Penicillinases	A	PC1	Serine	CV or PTZ	*Staphylococcus aureus*
Group 2b Penicillinases	A	TEM-1, TEM-2, SHV-1	Serine	CV or PTZ	*Enterobacteriaceae*
Group 2be ESBL	A	TEM-3, SHV-2, CTX-Ms, PER-1, VEB-1	Serine	CV or PTZ	*Escherichia coli, Klebsiella pneumoniae, K.oxytoca, Proteus mirabilis, Salmonella* spp.
Group 2 ber ESBL	A	TEM-50	Serine	Not inhibited by CV or PTZ	*Enterobacteriaceae*
Group 2 d	D	OXA-01, OXA-10		Variable with CV or PTZ	*Enterobacteriaceae*

Bush-Jacoby classification (2010)	Ambler Molecular class	Enzymes	Active site	Enzyme inhibitors	Found in
Group 2 de ESBL	D	OXA-11, OXA-15	Serine	Variable with CV or PTZ	*P.aeruginosa*
Group 2 df Carbapenemase	D	OXA-23, OXA-48	Serine	Variable with CV or PTZ	*Acinetobacter baumannii Enterobacteriaceae*
Group 2e ESBL	A	CepA	Serine	CV but not Aztreonam	*Proteae*
Group 2f Carbapenemase	A	KPC-2, SME-1, IMI-1	Serine	Variable with CV or PTZ	*Enterobacteriaceae*
Group 3 Metallo-carbapenemase	B	IMP-1, VIM-2, IND-1, L1	Zinc	EDTA	*P.aeruginosa, A.baumannii*
Group 4	Not included	Unknown			

PBA- Phenyl Boronic Acid DPA- Di picolinic Acid CV- Clavulanic acid
PTZ- Piperacillin-Tazobactam EDTA- Ethylene Diamine Tetra Acetate

Extended spectrum beta-lactamases (ESBL) and AmpC beta-lactamases are capable of hydrolyzing cephalosporins. ESBL belongs to Class A beta-lactamase of Ambler molecular classification while AmpC beta-lactamase belongs to ClassC. Carbapenems, β-lactams and β-lactamase inhibitor combinations such as piperacillin-tazobactam are the drugs active against ESBL and AmpC producing *Pseudomonas aeruginosa* isolates.

Nowadays, there is increased resistance to these drugs because of the emergence of metallo β-lactamases (MBL). In *Pseudomonas aeruginosa*, the

carbapenem resistance is predominantly mediated by metallo β-lactamases. These enzymes belong to Class B β-lactamase of Ambler classification. These enzymes can hydrolyse all classes of β-lactam antibiotics with the exception of monobactams (Aztreonam) and resist neutralization by β-lactamases inhibitor antibiotics.[11]

Plasmid mediated MBL genes spread rapidly to other species of gram-negative bacilli. In recent years, MBL genes have spread from *P. aeruginosa* to Enterobacteriaceae, and a clinical scenario appears to be developing that could simulate the global spread of extended-spectrum beta-lactamases. It is known that poor outcome occurs when patients with infections due to metallo β-lactamase producing organisms are treated with antibiotics to which the organism is completely resistant.[23] Hence, rapid detection of metallo β-lactamase production is important to modify therapy and to initiate effective infection control in preventing their dissemination.[24]

Acinetobacter baumannii

Acinetobacter species are ubiquitous, aerobic gram-negative bacteria that are widely distributed in soil and water and can be cultured from skin, mucous membranes, secretions, and the hospital environment. Acinetobacters often are commensals but cause nosocomial infection in immunocompromised patients.

Acinetobacter baumannii is the most commonly isolated species of clinical importance. *A.baumannii* has been isolated from various clinical specimens such as blood, sputum, skin, pleural fluid, and urine, usually in device-associated

infections. *Acinetobacter lwoffii, Acinetobacter johnsonii, Acinetobacter haemolyticus* are the other *Acinetobacter* species of low significance.[16]

CLASSIFICATION OF ACINETOBACTER SPECIES[17,22]

Acinetobacter baumannii belongs to the family *Moracellaceae*. Based on DNA hybridization, *Acinetobacter* species can be grouped into several genomospecies up to 25 types.

Table 3: Classification of *Acinetobacter* species

Genomospecies	Current designation
1	*A.calcoaceticus*
2	*A.baumannii*
3	*A.pittii*
4	*A.haemolyticus*
5	*A.junii*
6	Unnamed
7	*A.johnsonii*
8/9	*A.lwoffii*
10	*A.bereziniae*

MORPHOLOGY AND IDENTIFICATION

MICROSCOPY

They are strictly aerobic short, stout gram-negative coccobacilli, arranged singly or in pairs often appearing as diplococco-bacilli. Often they are gram-positive and gram variable; non-motile, non-sporing and some strains are capsulated.[17,18]

CULTURAL CHARACTERISTICS

On nutrient agar, after 24 hours the colonies are white or cream coloured, smooth, circular of 0.5 to 2 mm in diameter, translucent to opaque, convex with entire edges and never pigmented. On MacConkey agar, most strains grow well and produce a faint pink tint. The colonies are non-hemolytic on blood agar. Growth at 42ºC is a feature differentiating *A.baumannii* from *A.lwoffii.*[17,18]

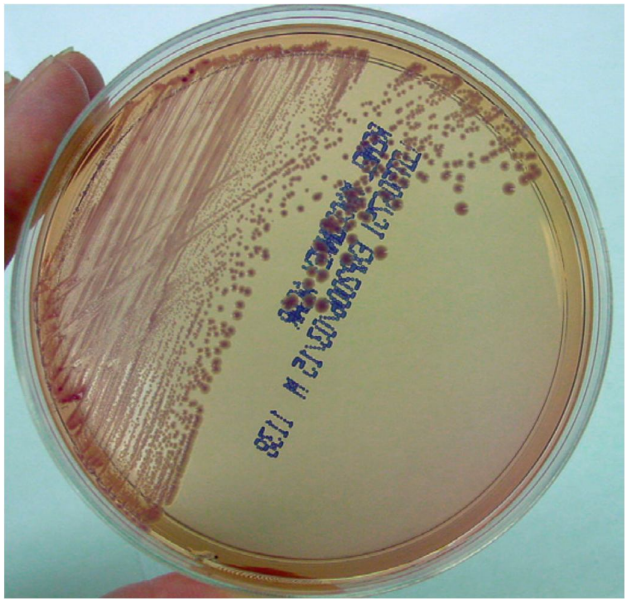


Fig:3 Colonies of *A.baumannii* on MacConkey agar –
Non-lactose fermenter with pink tint

BIOCHEMICAL REACTIONS

Catalase positive, oxidase negative, non-fermenting coccobacilli; saccharolytic - acidifies carbohydrates oxidatively (glucose, lactose), oxidatively uses 10% lactose (Hugh-Leifson's oxidation fermentation media), utilizes citrate,

indole negative, do not reduce nitrates to nitrite, some strains produce urease, dihydrolyses arginine.[14,17,18]

PATHOGENESIS

HOSPITAL ADAPTIVENESS

The role of the hospital environment as a reservoir for *A. baumannii* is supported by the fact that this organism can be recovered from patients and various hospital environmental sources during outbreaks. A number of studies show that particular strains can be isolated from the same hospital during a long period of time. The ability to survive under desiccative conditions as well as resistance to disinfectants and antimicrobials demonstrate how well *A. baumannii* can adapt and lead to long term persistence in the hospital environment.[25]

Table 4: Putative genes for hospital adaptiveness

Name of gene or protein	Function
csuC and *csuE*	Secretion and pili assembly Biofilm formation
$bla_{\text{PER-1}}$	β-lactamase production Associated with cell adhesiveness
Bap	Intercellular adhesion Biofilm maturation
pga	PNAG synthesis – adhesin for maintenance of biofilm structural stability

csuC, csuE – Chaperone-usher pili assembly system

$bla_{\text{PER-1}}$ – *Pseudomonas* Extended Resistant β-lactamase

Bap – biofilm associated protein gene

pga– poly-β-1,6- N-acetyl Glucosamine gene

VIRULENCE FACTORS[16,17]

Usually acinetobacters are not pathogenic, but they do have components that are capable of enhancing their virulence in debilitated individuals.

- **Endotoxin** - Endotoxin is a lipopolysaccharide (LPS) moiety in the outer membrane, in which the toxic lipid component, lipid A, is embedded. It induces inflammatory response that leads to tissue injury and responsible for the febrile response during septic episodes.
- **Outer membrane protein A (OmpA) –** mediates adhesion, invasion and cytotoxicity through mitochondrial damage.
- **Fimbriae -** capable of facilitating adhesion to human epithelial cells.
- **Polysaccharide capsule –** core virulence factor which limits the phagocytosis, aids the bacterium to survive under dry conditions.
- **Siderophores and iron-repressible outer membrane receptor proteins -** The ability of *A. baumannii* to grow under iron-deficient conditions is known to be associated with invasiveness.
- **Quorum sensing and biofilm formation**

CLINICAL SPECTRUM OF INFECTIONS

Acinetobacter baumannii is an environmental bacterium and has been isolated from opportunistic infections such as pneumonia, bacteraemia, meningitis, endocarditis, burn wound sepsis, urinary tract, eye and bone

infections. It is mostly associated with nosocomial infections, however community acquired infections has also been reported.

The risk factors include long term hospitalization in ICU (Intensive Care Unit), use of antibiotics, use of invasive diagnostic and therapeutic procedures, immunocompromised diseases, use of steroids, major surgery, burns, malignancy and severe underlying diseases. *A.baumannii* infections are mainly centered in the ICUs like respiratory, neurosurgical, neonatal and burns. Most common nosocomial infection include Ventilator associated pneumonia (VAP), Central line associated blood stream infections (CLABSI), Catheter associated urinary tract infections (CAUTI) and Surgical site infections (SSI).[14,17,26]

MECHANISM OF ANTIBIOTIC RESISTANCE

Acinetobacter baumannii is relatively resistant to almost all the available drugs and increased antimicrobial resistance has been implicated in nosocomial infections and hospital outbreaks.[1] *Acinetobacter baumannii* is intrinsically resistant to many antimicrobial agents like ampicillin, amoxicillin, amoxicillin-clavulanate, aztreonam, ertapenem, trimethoprim, chloramphenicol and fosfomycin.[21]

Unlike *Pseudomonas aeruginosa*, antimicrobial resistance in *Acinetobacter baumannii* is predominantly through acquired resistance mechanisms such as production of ESBL, Class A carbapenemases(bla_{SHV}, $bla_{TEM,}$ $bla_{GES,}$$bla_{KPC}$), Class B metallo β-lactamases (MBL – bla_{NDM}, bla_{VIM}, bla_{IMP}), Class C β-

lactamases (*Acinetobacter* derived cephalosporinases) and the most common Class D β-lactamases (*bla*$_{\text{OXA-23}}$ like, *bla*$_{\text{OXA-24}}$ like, *bla*$_{\text{OXA-51}}$ like,*blaOXA*-58 like).

Non-enzymatic mechanisms such as membrane impermeability by either loss of or decrease in expression of outer membrane proteins (*CarO*) or an increased expression of efflux pumps (*AdeABC*) also contributes to antimicrobial resistance in *Acinetobacter baumannii.*[1]

CARBAPENEM

HISTORICAL BACKGROUND

In the late 1960, as bacterial β-lactamases emerged and threatened the use of penicillin, the search for β-lactamase inhibitors began. By 1976, the first β-lactamase inhibitors were discovered; these were natural products produced by the Gram-positive bacterium *Streptomyces clavuligerus*. This was followed by the discovery of thienamycin in 1976, produced by *Streptomyces cattleya*. The term "Carbapenem" is defined as the 4:5 fused ring lactam of penicillins with a double bond between C-2 and C-3 but with the substitution of carbon for sulfur at C1.[27,28]

Thienamycin, an unstable carbapenem had inhibitory microbiological activity against Gram-negative bacteria, including *Pseudomonas aeruginosa*, anaerobes likes *Bacteriodes fragilis* and Gram-positive bacteria. Hence, years later, a more stable thienamycin derivative, known as Imipenem, was synthesized and approved for use in 1984. It became the first carbapenem approved for the treatment of complex microbial infections.[27,28,29]

However, imipenem was susceptible to deactivation by dehydropeptidase - 1 (DHP-1), found in the human renal brush border. Therefore, co-administration with an inhibitor, cilastatin or betamipron in the ratio of 1:1 with imipenem prevented hydrolysis by DHP-1 and reduced nephrotoxicity. Along the journey to the discovery of more-stable carbapenem with extended spectrum, the other currently available compounds such as meropenem, biapenem, ertapenem, and doripenem were developed with the addition of a methyl group to the 1-β position. Meropenem was the first carbapenem with the 1-β-methyl group which renders this antibiotic stable to DHP-1.[27,30]

CLASSIFICATION

Carbapenems are classified into three groups.

- Group 1 Carbapenems are defined as broad-spectrum agents that have limited/no activity against non- fermentative Gram-negative bacilli (NFGNB) and are most suited for use in treating infections caused by Enterobacteriaceae - e.g. Ertapenem.
- Group 2 Carbapenems are broad-spectrum agents that are active against NFGNB and are particularly useful in treating nosocomial infections – e.g. Imipenem, Meropenem, Doripenem.
- Group 3 Carbapenems which include agents with activity against methicillin-resistant *Staphylococcus aureus* (MRSA), such as Razupenem (PZ-601).[27,31]

MECHANISM OF ACTION

Carbapenems act by inhibiting the synthesis of the peptidoglycan layer of the bacterial cell wall. Carbapenems are not easily diffusible through the bacterial cell wall. Carbapenems enter Gram-negative bacteria through outer membrane proteins (OMP), also known as porin proteins. Carbapenems act by inhibiting peptide cross-linking as well as other peptidase reactions. Thereby, the peptidoglycan weakens, and the cell bursts due to osmotic pressure.[28,29,32]

ACTIVITY OF CARBAPENEMS

Carbapenems have a broad spectrum of antimicrobial activity and are rapidly bactericidal agents because they bind with high affinity PBPs of Gram-negative bacteria. Carbapenems are the drug of choice for the treatment of infections caused by ESBL producing *Enterobacteriaceae.* Carbapenems (except ertapenem) are active against clinically significant gram-negative non-fermenters such as *P. aeruginosa*, *Burkholderia cepacia* and *Acinetobacter* spp. They also retain activity against streptococci, methicillin-sensitive staphylococci, *Neisseria* and *Haemophilus.*[27,33]

Unlike most other broad-spectrum antibiotics, carbapenems are active against most Gram-positive and Gram-negative anaerobes. Carbapenem-resistant bacteria include ampicillin-resistant *Enterococcus faecium,* methicillin-resistant *Staphylococci, Stenotrophomonas maltophilia* and some isolates of *Clostridium difficile.*[27,33]

CARBAPENEM RESISTANCE

Carbapenems are the drugs of choice in the treatment of infections caused by multidrug resistant *P. aeruginosa* and *A. baumannii*. Emergence and spread of carbapenem resistance limits therapeutic options to polymyxins and tigecycline. Resistance to carbapenems is mediated by lack of drug penetration (i.e., porin mutations and efflux pumps) and / or carbapenem hydrolysing beta lactamase enzymes (carbapenemases).

PREVALENCE OF CARBAPENEM RESISTANCE

The rates of carbapenem resistance in glucose non-fermenting gram-negative bacilli have been gradually increasing worldwide over the last 10 years and vary geographically. The highest burden of carbapenem resistance among gram-negative healthcare associated infections in the US as reported by the NHSN from 2009-2010 was observed 86 among *A. baumannii* (62.6%) and *P. aeruginosa* (26.1%) in comparison to CRE where carbapenem resistance was highest among *Klebsiella pneumoniae* at 12.8%.[34,35] In India, carbapenem resistance ranges from 10.9 - 69% in *Pseudomonas aeruginosa* and 9.1-100% in *Acinetobacter baumannii* has been reported among various patient populations in different sample types, predominantly from respiratory specimens and pus samples.[7,8,9,10]

Carbapenem resistant *P.aeruginosa* infection not only increase mortality, but it is also associated with increased morbidity. Carbapenem–resistant *Acinetobacter baumannii* infection usually occurs in severely ill patients in the

ICU, therefore the associated crude mortality rate is high. Crude mortality rates of 30 - 75% have been reported for nosocomial pneumonia caused by *A. baumannii*.

CARBAPENEM RESISTANCE IN PSEUDOMONAS AERUGINOSA

Carbapenem resistance mechanisms in *P.aeruginosa* include

1. Production of carbapenem hydrolyzing enzymes (carbapenemases)
2. Increased production of Amp C chromosome encoded cephalosporinases
3. Reduced outer membrane porin – Opr D expression
4. Overexpression of efflux pumps

CARBAPENEMASES IN PSEUDOMONAS AERUGINOSA

Carbapenemases mediating carbapenem resistance in *Pseudomonas aeruginosa* belong to Ambler Class A and Class B β-lactamases.

Class A carbapenamases in *P. aeruginosa*

The first report of KPC-producing *P. aeruginosa* isolates was described in three genetically related isolates from Colombia in 2007. The spread of bla_{KPC} into different genera is most likely due to its presence within mobile genetic elements on plasmids of various sizes[36].

Class B metallo beta-lactamases in *P. aeruginosa*

In general, carbapenem resistance in *P. aeruginosa* attributed to β-lactamases is due to MBL. Production of MBL by *P. aeruginosa* leads to resistance to all betalactams except the monobactams such as aztreonam. The most common MBL families include the VIM, IMP, NDM, SPM, GIM and SIM

enzymes, which are located within a variety of integron structures, where they have been incorporated as gene cassettes. When these integrons become associated with plasmids or transposons, transfer of this resistance between bacteria is readily facilitated.

Since their initial discoveries, SPM, GIM, and SIM metallo-β-lactamases have not spread beyond their countries of origin. However, VIM and IMP continue to be detected worldwide, with an overall trend of these two MBLs moving beyond *P. aeruginosa* and into the *Enterobacteriaceae.* The prevalence of MBL in India has ranged from 7% to 65% among carbapenem-resistant *P.aeruginosa* and *bla* $_{VIM}$ type was the most common.[36]

EFFLUX MEDIATED CARBAPENEM RESISTANCE IN *P. AERUGINOSA*

Active efflux is an important non-enzymatic mechanism of β-lactam resistance in *P. aeruginosa*. Efflux also contributes to the development of multiple resistances to all antipseudomonal antibiotics and is mediated by four genetically different three component efflux systems that belong to the resistance–nodulation–division (RND) family: MexA–MexB–OprM, MexC–MexD–OprJ, MexE–MexF–OprN and MexX–MexY–OprM.[37]

PORIN DEFECTS IN *PSEUDOMONAS AERUGINOSA*

Loss of the OprD porin in *P. aeruginosa* is an important mechanism associated with imipenem resistance. The *P. aeruginosa* porin Opr D is a substrate-specific porin that has been shown to facilitate the diffusion of basic amino acids, small peptides that contain these amino acids, and carbapenems into

the cell. Loss of Opr D production is likely due to inactivation of the Opr D gene. Loss of Opr D does not confer resistance to β-lactams other than the carbapenems. Mutational loss of Opr D is frequent during Imipenem therapy.

The impact of Opr D deficiency on the potency of these carbapenems does not always push the MICs above the susceptible breakpoint, and additional resistance mechanisms (efflux pump and/or carbapenemase) may be required to provide resistance to the carbapenems.[37,38]

CARBAPENEM RESISTANCE IN *ACINETOBACTER BAUMANNII*

Carbapenem resistance in *A. baumannii* is due to a variety of combined mechanisms such as hydrolysis by β lactamases, alterations in outer membrane protein and penicillin binding proteins and increased activity of efflux pumps.

CARBAPENEMASES IN *ACINETOBACTER BAUMANNII*

Of the β-lactamases, those with carbapenemase activity are most concerning and include the serine oxacillinases (Ambler class D OXA type) and the MBLs (Ambler class B). These carbapenemases are of greatest concern as they are encoded by genes which are transmissible.

OXA Carbapenemases in *Acinetobacter baumannii*

The first identified OXA type enzyme with carbapenem hydrolysing activity was from *A.baumannii* strain isolated in 1985 from Scotland and was originally named ARI (Acinetobacter Resistant to Imipenem),but was renamed as *bla* $_{\text{OXA -23}}$. *Bla* $_{\text{OXA -23}}$ cluster (*bla* $_{\text{OXA -23,27,49}}$) now contribute to carbapenem

resistance in *A. baumannii* globally. The *bla* $_{\text{OXA-23}}$, *bla* $_{\text{OXA-24}}$ and *bla* $_{\text{OXA-58}}$ like enzymes are plasmid / chromosomally encoded which explains their widespread distribution.[26,39]

The *bla* $_{\text{OXA-51}}$ -like gene cluster is unique in that it is naturally occurring in *A.baumannii*. Similar to other class D enzymes, they have greater affinity for imipenem than meropenem. Their role in carbapenem resistance is related to the presence of an insertion sequence IS*Aba1*, situated upstream possibly providing a promoter for hyper production of beta lactamase genes.[40]

Class B Metallobetalactamase in *Acinetobacter baumannii*

IMP-like, VIM-like, SIM-1 and NDM are the MBLs identified in *A. baumannii*. The IMP and VIM variants confer a high level of carbapenem resistance in *A. baumannii*.

NON ENZYMATIC MECHANISMS IN *ACINETOBACTER BAUMANNII*

β-lactam resistance, including carbapenem resistance, has also been ascribed to non-enzymatic mechanisms, including changes in outer membrane proteins, multidrug efflux pumps, and alterations in the affinity or expression of penicillin binding proteins (PBP).[41]

Outer membrane proteins

A. baumannii possesses OMPs that play a role in carbapenem resistance. By reduction of transport into the periplasmic space via changes in porins or OMPs, access to PBP is reduced. With less β-lactam entering the periplasmic

space, the weak enzymatic activity of the β-lactamase is amplified. Many outbreaks of infection with imipenem-resistant *A. baumannii* are due to porin loss. Quale *et al.* found that carbapenem-resistant isolates of *A. baumannii* had reduced expression of 47-, 44-, and 37-kDa OMPs.[41]

Efflux pump in *Acinetobacter baumannii*

The resistance-nodulation-division (RND) family-type pump AdeABC is the best studied thus far and has a substrate profile that includes β-lactams (including carbapenems), aminoglycosides, erythromycin, chloramphenicol, tetracyclines, fluoroquinolones and trimethoprim. AdeABC has a three-component structure: AdeB forms the trans-membrane component, AdeA forms the inner membrane fusion protein, and AdeC forms the OMP. AdeABC is chromosomally encoded.[26,42]

PBP in *Acinetobacter baumannii*

Modification of PBPs as a source of imipenem resistance in *A. baumannii* has been investigated only rarely.[43]

TREATMENT

Of the 6 famous ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter species, Pseudomonas aeruginosa*, and *Enterobacter* species), *P.aeruginosa* and *A.baumannii* are the predominant pathogens causing nosocomial infections. World Health Organization (WHO) has categorized Carbapenem resistant

Pseudomonas aeruginosa and *Acinetobacter baumannii* as Priority 1- Critical organisms for the research and development of newer antibiotics.[12]

Recognizing carbapenemase expression is the key to the appropriate management of infections caused by carbapenem-resistant isolates. Unusually elevated MICs to carbapenems should arouse suspicion for a carbapenem-resistant isolate and preclude the use of carbapenems even if the MICs do not exceed the breakpoints for resistance. As with ESBL-producing organisms, carbapenemase-producing strains are likely to exhibit simultaneous resistance to aminoglycosides and fluoroquinolones.

ANTIBIOTICS OF CHOICE

Aztreonam

It is stable to metallo-carbapenemases, including IMP, VIM and NDM. However, for isolates that also co-produce AmpC or ESBL, aztreonam is ineffective.[44]

Sulbactam

Sulbactam is active against *A.baumannii* by inhibiting PBP-2. In most countries it is available as a co-formulation with ampicillin. Sulbactam is useful in the treatment of carbapenem resistant *A.baumannii* infections in combination with colistin.[45]

Tigecycline

Tigecycline, a tetracycline analogue is the first glycylcycline to be launched for clinical use. It acts by inhibiting the protein synthesis in the bacterial cell by binding to the 30S subunit of the ribosome. Its capacity to penetrate into various tissues makes it useful in the treatment of infections of the skin and soft tissues as well as intra-abdominal infections, whereas its low serum concentrations compromise its use in bloodstream infections. It is not useful in treatment of nosocomial pneumonia as indicated by poor results in the study of ventilator associated pneumonia. It is affected by the intrinsic multidrug efflux pumps of *P.aeruginosa*. Therefore, not useful in the treatment of infections caused by *P.aeruginosa*.[45]

Polymyxin

Given limited therapeutic options, clinicians have returned to the use of polymyxin B or polymyxin-E (colistin) for the most *carbapenem* resistant gram-negative infections. Polymyxin B differs from colistin by only one aminoacid. These drugs act by disturbing the bacterial cell *membrane*, thus increasing permeability, leading to cell death.[26,46,47]

Fosfomycin

Fosfomycin inhibits bacterial cell wall synthesis, thereby exhibiting bactericidal activity against gram-positive and gram-negative pathogens. Fosfomycin is useful for the treatment of uncomplicated urinary tract infections at

a single oral dose. The emergence of resistance among *GNB* has sparked new interest in using fosfomycin to treat infections caused by MDR isolates.[26,46,47]

Extended-Infusion Strategy for β-Lactams

Carbapenems have also been evaluated in extended-infusion regimens. Lengthening meropenem infusions from 30 minutes to 3 hours was found to be advantageous with isolates of *P. aeruginosa* and *A.baumannii* with intermediate resistance. This benefit was not observed with resistant isolates having very high MIC.[26,46,47]

Combination of a carbapenem with another active agent, preferentially an aminoglycoside or colistin could lower mortality provided that the Minimum Inhibitory Concentration of carbapenem for the infecting organism is up to 4 µg/ml – and up to 8 µg/ml and the drug is administered in a high-dose/prolonged-infusion regimen. In cases where the MICs for carbapenems are not available or are higher than 8 µg/ml, this class of drugs should not be used as part of a combination regimen to avoid further selection of resistance.

INFECTION PREVENTION MEASURES

Centre for Disease Control and prevention (CDC) recommendations for preventing transmission of Carbapenem resistant organisms include

Laboratory detection of Carbapenem resistance

Accurate detection of carbapenem resistance is the first step in prevention.

Recognizing Carbapenem resistance cases

It is important for health care facilities to understand how far carbapenem resistance is prevalent in their institutions. In the investigations conducted by the CDC, failure in recognizing carbapenem resistant infections when they first occur has resulted in a missed opportunity to intervene before these organisms are transmitted more widely.

Based on the current recommendations for the control of multidrug-resistant organisms (MDROs), in areas where carbapenem resistant organisms are not endemic, acute care facilities review microbiology records for the preceding 6–12 months to determine whether carbapenem resistant organisms have been isolated at the facility. If previously unrecognized cases are identified, a round of surveillance cultures (i.e., a point prevalence survey) in high-risk areas (e.g., ICUs or wards where previous cases have been detected) should be considered to identify unrecognized cases.

In addition, facilities should ensure a system is in place to notify infection control team when carbapenem resistant organisms are identified in the laboratory. All identified carbapenem resistant organisms case-patients should be placed on contact precautions, and patient cohorting and use of dedicated staff is also recommended for these patients.[26,48]

Surveillance cultures

If previously unrecognized carbapenem resistant cases or hospital-onset infections are identified via either clinical cultures or point prevalence surveys,

facilities should consider surveillance cultures from patients with epidemiologic links to carbapenem resistant case-patients.

The goal of these cultures is to identify patients colonized with additional unrecognized carbapenem resistant organisms who are a potential source for transmission.[26,48]

Antimicrobial Stewardship and minimizing devices

Antimicrobial stewardship is an important part of efforts to control MDROs. However, multiple antimicrobial classes have been identified as possible risk factors for infection or colonization with carbapenem resistant organisms. Therefore, antimicrobial stewardship will be most effective if efforts are directed toward an overall decrease in antimicrobial use rather than targeting a specific antimicrobial class. Limiting use of invasive devices is another important intervention for prevention.[26,48]

Antibiotic cycling

Antibiotic cycling or rotation is the scheduled substitution of a class of antibiotics with a different class that exhibits a comparable spectrum of activity. This substitution may be followed after a fixed interval by any number of substitutions but the cycle must be repeated with re-introduction of the original class/drug. The duration of each cycle is based on either local susceptibility patterns or a predetermined time period.[49]

Prevention beyond Acute Care and Role of Public Health

Although much of the effort for prevention has focused on acute care facilities, non-acute care settings also provide care for patients colonized or infected with these organisms. Limiting prevention efforts to acute care settings fails to take into account the presence of MDROs across different health care settings. Broadening the approach to prevention requires employing setting-specific infection prevention strategies in all health care facilities but also requires a method for enhanced communication to ensure that proper infection-control practices are continued when patients are transferred between levels of care.[26,48]

COLISTIN IN THE TREATMENT OF CARBAPENEM RESISTANT *PSEUDOMONAS AERUGINOSA* AND *ACINETOBACTER BAUMANNII*

Colistin, a polymyxin antibiotic (polymyxin E), was first discovered in the 1940s but was not used clinically until the late 1950s. Historically, colistin was used to combat infections caused by the gram-negative bacteria. Reports of nephrotoxicity and neurotoxicity, however, deterred physicians from using the antibiotic, especially with the emergence of other less toxic antibiotics (e.g., aminoglycosides). Hence between the 1970s and 1990s, colistin was not used often.[50]

Nowadays, the lack of treatment options for MDR bacteria such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* has led to the reemergence of colistin as an antimicrobial therapy.

Colistin is available in two forms, colistin sulfate and colistimethate sodium, administered topically and parenterally, respectively. Both forms can be inhaled.

MECHANISM OF ACTION

Colistin disrupts the outer membrane and releases lipopolysaccharides. Change in the permeability of the bacterial membrane leads to leakage of the cell content and subsequently cell lysis and death. Colistin also has the ability to bind and neutralize the lipopolysaccharide molecule of bacteria, giving it anti-endotoxin activity.[51,52]

SPECTRUM OF ACTIVITY

Colistin has a narrow antibacterial spectrum of activity, predominantly against gram-negative isolates. Most significantly, it displays in vitro activity against MDR gram-negative pathogens such as *A. baumannii, P. aeruginosa*, and *K. pneumoniae.* Colistin also has activity against other isolates such as members of *Enterobacteriaceae - Escherichia coli, Salmonella* species, *Shigella* species, *Stenotrophomonas maltophilia, Haemophilus influenzae, Bordetella pertussis*, and *Legionella pneumophila*.

COLISTIN RESISTANCE

Although colistin is often considered as a reliable agent to treat carbapenem resistant *P.aeruginosa* and *A. baumannii*, reports of colistin resistant strains are on the rise. Recent studies have shown varying rates of resistance as well as the occurrence of hetero-resistant strains. Hetero-resistance occurs when

subpopulations within the strain exhibit reduced susceptibility although the overall MIC is not altered. This makes detection of resistant subpopulations impossible with MIC alone.

According to *Mohanty et al*, in India, the prevalence of colistin resistance was found to be 6% in *A.baumannii* and about 8% in *P.aeruginosa*. *Taneja et al* reported the colistin resistance in *A.baumannii* was about 3.5%.[4,53] *Baurah FK et al* in his study in 2014 reported that *P.aeruginosa* isolates were 100% susceptible to colistin.[54]

MECHANISM OF RESISTANCE

Resistance to colistin can develop through adaptive or mutational mechanisms. Mechanism of resistance includes changes in the structure of the bacterial negatively charged surface lipopolysaccharides and lipid A. These modifications occur as a result of the activation of the PmrA-PmrB system, which is regulated by the PhoP-PhoQ system.[50,51,52]

Colistin resistance is recently due to a plasmid mediated gene *mcr-1* in *Escherichia coli*. It was first isolated in China in 2015; this is not yet identified in other species. Because of the possibility of the transfer of this gene from one bacterial species to other, their global distribution and close monitoring of colistin resistance is warranted.[55] SENTRY Antimicrobial Surveillance Program is tracing the global spread of *mcr-1* gene since it was identified.

The development of colistin resistance has also been linked to inadequate dosing. This highlights the importance of dose optimization, especially in critically ill patients with MDR bacterial infections. Although higher doses appear beneficial, the lack of pharmacodynamic and pharmacokinetic data regarding colistin makes determination of appropriate dosing difficult. Colistin remains an essential alternative for most MDR gram-negative infections; however, cases of resistant strains should be a cause of great concern.

Materials & Methods

MATERIALS AND METHODS

Place of study:

This study was conducted at Institute of Microbiology, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai-3.

Study period:

This study was conducted for one year from April 2016 to March 2017

Study type:

Descriptive study

Ethical consideration:

Approval was obtained from the Institutional Ethics Committee before starting the study. Informed written consent was obtained from all the patients participated in this study. All patients satisfying the inclusion criteria were included.

Statistical analysis:

Statistical analyses were carried out using Statistical Packages for Social Sciences (SPSS). The proportional data of this cross sectional study were analyzed using Pearson's Chi Square analysis test.

Study population:

A total of 150 (75 *P.aeruginosa* and 75 *A.baumannii*) clinically significant, consecutive, non-repetitive isolates of *Pseudomonas aeruginosa* and

Acinetobacter baumannii were included in this study. The isolates were obtained from clinical specimens including sputum, endotracheal aspirate, bronchial wash, pleural fluid, ascitic fluid, peritoneal dialysis fluid, blood, urine, cerebrospinal fluid and wound swab of patients admitted in various wards.

Inclusion Criteria:

1. Clinically significant, consecutive, non-repetitive isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were included. The significance of the isolates were based on two or more of the following criteria – clinical history, presence of organism in Gram stain, presence of intracellular forms of the organism and pure growth in culture with a significant colony count wherever applicable.
2. Patient aged more than 18 years.

Exclusion Criteria:

1. Isolates of repeated samples from the same patient were not included in the study.
2. Patients with colonization of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* with no apparent clinical illness.

Preliminary identification of the isolates of *Pseudomonas aeruginosa* was done based on the following characteristics:

1. **Colony Morphology:**

   On Nutrient agar – Opaque, irregular colonies with metallic sheen and blue green diffusible pigment.

   On Blood agar – Spreading and flat colonies with serrated edges; with or without hemolysis.

   On MacConkey Agar – Non-lactose fermenting colonies.

2. The isolates were subjected to preliminary tests like Gram stain, catalase test, oxidase test and motility by hanging drop method.

3. The isolates which were gram-negative bacilli, catalase positive, oxidase positive and motile were subjected to biochemical reactions for further confirmation.

4. The biochemical reactions performed were Hugh – Leifson's oxidative fermentative test, Indole production using Kovac's reagent, Triple sugar iron medium for sugar fermentation and hydrogen sulphide production, citrate utilization test, urea hydrolysis test, Moeller's decarboxylation (arginine dihyrolysis).

Table 5: Biochemical reactions of *Pseudomonas aeruginosa*

OF Glucose test	Oxidative
Indole test	Negative
Triple sugar iron media	Alkaline slant/Alkaline butt, no gas, no H_2S
Citrate test	Positive
Urea hydrolysis	Variable
Growth at 42^{o}C	Positive
Pyocyanin/Pyoverdin	Present
Arginine	Dihydrolysed
Polymyxin B (300U)	Sensitive

Preliminary identification of the isolates of *Acinetobacter baumannii* was done based on the following characteristics:

1. Colony Morphology:

   On Blood agar – Small, circular, convex, smooth colonies with or without hemolysis.

   On MacConkey Agar – Non-lactose fermenting colonies sometimes with pinkish hue.

2. The isolates were subjected to preliminary tests like Gram stain, catalase test, oxidase test and motility by hanging drop method.
3. The isolates which were gram-negative coccobacilli, catalase positive, oxidase negative and non-motile were subjected to biochemical reactions for further confirmation.

4. The biochemical reactions performed were Hugh – Leifson's oxidative fermentative test, Nitrate reduction test, Indole production using Kovac's reagent, triple sugar iron medium for sugar fermentation and hydrogen sulphide production, citrate utilization test, urea hydrolysis test.

Table 6: Biochemical reactions of *Acinetobacter baumannii*

OF (Glucose) test	Oxidative
Nitrate reduction test	Negative
Indole test	Negative
Triple sugar iron media	Alkaline slant/Alkaline butt, no gas, no H_2S
Citrate test	Positive
Urea hydrolysis test	Negative
Growth at 42^oC	Positive
10% OF Lactose	Fermented

ANTIMICROBIAL SENSITIVTY TESTING

Disc Diffusion Method:

Antimicrobial sensitivity testing was performed for all the isolates by Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates.

Three to four colonies were inoculated in peptone water and incubated for two hours at 37ºC, to bring the organism to logarithmic phase. The turbidity of the suspension was adjusted to 0.5 McFarland standards. Within fifteen minutes of

preparation of the suspension, a sterile cotton swab was immersed in the suspension and the excess suspension is removed by rotating the swab against the wall of the test tube. A lawn culture of the inoculum was made by streaking the swab over the surface of the plate in three directions. After about 10 to 15 minutes, the antibiotic discs were placed, five on each plate and incubated at 37ºC for 20 to 24 hours.

Zone of inhibition of bacterial growth around the antibiotic discs were measured using the Himedia scale. Interpretations were made using the Clinical and Laboratory Standards Institute, USA guidelines – January 2016, M100S.

Table 7: Panel of Antibiotics for *Pseudomonas aeruginosa* isolates and interpretative criteria

ANTIBIOTICS	ZONE OF INHIBITION (mm)		
	SENSITIVE	INTERMEDIATE	RESISTANT
Ceftazidime (30µg)	≥18	15-17	≤14
Piperacillin-Tazobactam (100/10 µg)	≥21	15-20	≤14
Cefepime (30 µg)	≥18	15-17	≤14
Gentamicin (10 µg)	≥15	13-14	≤12
Amikacin (30 µg)	≥17	15-16	≤14
Ciprofloxacin (5 µg)	≥21	16-20	≤15
Meropenem (10 µg)	≥19	16-18	≤15
Imipenem (10 µg)	≥19	16-18	≤15

Table 8: Panel of Antibiotics for *Acinetobacter baumannii* isolates and interpretative criteria

ANTIBIOTICS	ZONE OF INHIBITION (mm)		
	SENSITIVE	INTERMEDIATE	RESISTANT
Ceftazidime (30µg)	≥18	15-17	≤14
Piperacillin-Tazobactam (100/10 µg)	≥21	18-20	≤17
Tetracycline (30 µg)	≥15	12-14	≤11
Trimethoprim/Sulfamethoxazole (1.25/23.75 µg)	≥16	11-15	≤10
Gentamicin (10 µg)	≥15	13-14	≤12
Amikacin (30 µg)	≥17	15-16	≤14
Ciprofloxacin (5 µg)	≥21	16-20	≤15
Meropenem (10 µg)	≥18	15-17	≤14
Imipenem (10 µg)	≥22	19-21	≤18

MINIMUM INHIBITORY CONCENTRATION (MIC) OF IMIPENEM

MIC of Imipenem was determined by Epsilometer test (E-test) for all the imipenem intermediate and resistant isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

A predefined stable imipenem (Biomerieux) non-porous plastic E-strip of 5mm width and 60mm length (with concentration gradient ranges from 0.02 to 32µg/ml) is applied on to 0.5 McFarland standard suspension inoculated Mueller-Hinton agar plate, incubated at 37ºC for 20-24 hours. After incubation whereby bacterial growth becomes visible, a symmetrical inhibition ellipse centered along

the strip is formed. The MIC value is read from the scale in terms of µg/ml where the point of ellipse intersects the strip.

The same MIC interpretative criteria recommended in CLSI guidelines 2016 for broth dilution method were applied for E-test method.

Table 9: MIC interpretative criteria for Imipenem

Organism	Minimum Inhibitory Concentration (MIC) - µg/ml		
	Sensitive	**Intermediate**	**Resistant**
Pseudomonas aeruginosa	≤2	4	≥8
Acinetobacter baumannii	≤2	4	≥8

According to CDC-NHSN, isolates showing resistance to at least one agent in three or four groups of antibiotics (cephalosporins, carbapenems, fluoroquinolones and aminoglycosides) were considered as Multi-Drug Resistant (MDR) in this study.

DETECTION OF ANTIMICROBIAL RESISTANCE

Phenotypic screening methods

All the isolates included in this study were subjected to Extended Spectrum Beta-lactamase (ESBL) screening test using ceftazidime (30µg) and/or cefepime (30µg), AmpC beta-lactamase screening test using cefoxitin (30µg) and carbapenemase screening test using imipenem (10µg) and meropenem (10µg) discs. The isolates which were positive in the screening test were subjected to respective confirmatory tests using appropriate antibiotic discs.

Phenotypic confirmatory test for ESBL production- Combined disc method[3,56]

In this method, a lawn culture of the test isolates was made as for disc diffusion method. Ceftazidime (30µg) and ceftazidime-clavulanic acid (30µg/10µg) discs- Himedia, were placed at a distance of 20mm centre to centre on the Mueller-Hinton agar plate, incubated at 37ºC for 20-24 hours. The test isolate was considered to produce ESBL if the zone of inhibition around the ceftazidime-clavulanic acid disc was ≥5mm that the zone around ceftazidime disc alone.

Phenotypic test for AmpC detection[3,57]

The isolates which were resistant to cefoxitin [(30µg) <18mm] were considered as AmpC screening test positive. AmpC production was confirmed by placing cefoxitin (30µg) and cefoxitin-phenylboronic acid (30/400µg) at a distance of 20mm on the Muller-Hinton agar plate. The test isolate that demonstrated a zone of inhibition of ≥5mm around cefoxitin-inhibitor than that around the cefoxitin alone was considered as AmpC producer.

Carbapenemase detection by Modified Hodge Test (MHT)[58,59]

Isolates positive for screening test in the disc diffusion (resistant to carbapenems) was further processed by modified Hodge test to detect carbapenemase production.

0.5 McFarland standard suspension of *E.coli* ATCC 25922 was prepared in nutrient broth or saline and diluted 1:10 in saline or broth. A lawn culture of 1:10 dilution *E.coli* ATCC 25922 was done on to a Mueller Hinton Agar plate and allowed to dry for 3-5 minutes. A 10µg meropenem disc is placed in the center of the test area. In a straight line, the test organism was streaked from the edge of the disc to the edge of the plate and incubated at 37^0C for 16-20 hours.

Interpretation

Enhanced growth (Clover-leaf indentation) = Positive

No enhanced growth = Negative

Metallo beta-lactamase (MBL) detection by combined disc method

Metallo-β-Lactamase production for the carbapenem resistant isolates was screened by imipenem (10µg) –EDTA (750µg), meropenem (10µg) – EDTA (930µg) and ceftazidime (30µg) –EDTA (930µg) combined disc method. An increase in zone size of ≥ 7 mm around Inhibitor combination disc compared to disc without inhibitor was considered as MBL positive.[36]

COLISTIN SUCEPTIBILITY FOR THE CARBAPENEM RESISTANT ISOLATES

Colistin MIC was determined for carbapenem resistant isolates by Epsilometer test (E-test) using colistin E-strips of concentration gradient (0.016 to 256µg/ml (Biomerieux).

Table 10: MIC interpretive criteria for Colistin according to CLSI guidelines, 2016

Organism	Minimum Inhibitory Concentration (MIC) - µg/ml		
	Sensitive	**Intermediate**	**Resistant**
Pseudomonas aeruginosa	≤2	-	≥4
Acinetobacter baumannii	≤2	-	≥4

MOLECULAR CHARACTERIZATION

The Carbapenem resistant *Pseudomonas aeruginosa* isolates which were positive for MBL production and carbapenem resistant *Acinetobacter baumannii* isolates which were positive for carbapenemase production by modified Hodge test were subjected to conventional Polymerase Chain Reaction (PCR) for the detection bla_{VIM}/bla_{NDM-1} and bla_{OXA-23} genes respectively.

Bacterial DNA Purification

1. 1ml of overnight broth culture was centrifuged at 6000rpm for 5min. Supernatant was discarded and pellet was suspended in 0.2ml PBS.
2. 180µl of Lysozyme digestion buffer and 20µl of Lysozyme [10mg/ml] were added to the pellet and incubated at 37ºC for 15min.
3. 400µl of Binding buffer, 5µl of internal control template and 20µl of Proteinase K were added and mixed well by inverting several times; incubated at 56ºC for 15min. 300µl of Ethanol was added and mixed well.

4. The entire mixture is transferred into the PureFast® spin column and centrifuged for 1 min. Flow-through was discarded and the column is placed back into the same collection tube.
5. 500µl Wash buffer-1 was added to the PureFast® spin column and centrifuged for 30-60 seconds and the column is placed back into the same collection tube after discarding the flow through.
6. Then 500µl Wash buffer-2 was added to the PureFast® spin column and centrifuged for 30-60 seconds and the column is placed back into the same collection tube after discarding the flow through; centrifuge for additional 1 min.This step is essential to avoid residual ethanol.
7. The PureFast® spin column was transferred into a fresh 1.5 ml micro-centrifuge tube. Then, 100µl of Elution Buffer was added to the center of PureFast® spin column membrane; incubated for 1 min at room temperature and centrifuged for 2 min.
8. The column was discarded and the purified DNA was stored at -20°C. Quality of extracted DNA was checked by loading in 1% agarose gel and 5µl of extracted DNA was used for PCR amplification.

PCR Procedure:

PCR was done at HELINI BIOMOLECULES, Neelankarai, Chennai

1. Reactions set up as follows:

Components Quantity

HELINI RedDye PCR Master Mix 10µl

HELINI Ready to use – OXA23 gene Primer Mix / VIM gene primer mix/ NDM-1 gene primer mix 5µl

Purified Bacterial DNA 5µl

Total volume 20µl

2. Reaction components were mixed gently; placed into PCR machine and programmed as

Initial Denaturation: 94ºC for 5 min

Denaturation: 94ºC for 30sec

Annealing: 58ºC for 30sec } 35 cycles

Extension: 72ºC for 30sec

Final extension: 72º C for 5 min

Loading

2% agarose gel was prepared. [2gm of agarose in 100ml of 1X TAE buffer]; electrophoresis was done at 50V till the dye reaches three fourth distances and the bands were observed in UV Transilluminator.

Agarose gel electrophoresis

1. 2% agarose gel was prepared. (2gm agarose was mixed in 100ml of 1X TAE buffer and melted using microoven). When the agarose gel temperature was around 60ºC, 5µl of Ethidium bromide was added. Warm agarose solution was poured slowly into the gel platform and the gel was kept undisturbed till the agarose solidifies.
2. 1XTAE buffer was poured into submarine gel tank and the gel is placed.

3. PCR Samples were loaded after mixed with gel loading dye along with 10µl HELINI 100bp DNA Ladder. [100bp, 200bp, 300bp, 400bp, 500bp, 600bp, 700bp, 800bp, 900bp,1000bp and 1500bp]
4. Electrophoresis was done at 50V till the dye reaches three fourth distances and the bands were observed in UV Transilluminator.
5. PCR products were confirmed by the band formation of corresponding product size.

   Product size for bla_{OXA-23} – 453 bp

   Product size for bla_{VIM} – 480 bp

   Product size for bla_{NDM-1} – 475 bp

Results

RESULTS

In this descriptive study, 75 clinically significant, consecutive, non-repetitive *Pseudomonas aeruginosa* and 75 *Acinetobacter baumannii* isolates collected from various clinical specimens during April 2016 to March 2017 were included.

Table 11: Distribution of *Pseudomonas aeruginosa* isolates among various clinical samples (n=75)

Clinical Samples	No.of isolates (%)
Pus	32 (42.7%)
Urine	13 (17.3%)
Respiratory specimens (endotracheal aspirate, sputum, bronchial wash)	25 (33.3%)
Fluids (Peritoneal dialysis fluid, pleural fluid)	3 (4%)
Devices (catheter tip)	2 (2.7%)

The vast majority of *Pseudomonas aeruginosa* were isolated from pus samples. These samples were predominantly from patients with surgical site infections and diabetic foot infection.

Table 12: Distribution of *Acinetobacter baumannii* isolates among various clinical samples (n=75)

Clinical Samples	No .of isolates (%)
Pus	21 (28%)
Urine	18 (24%)
Respiratory specimens (endotracheal aspirate, sputum)	22 (29.3 %)
Blood	6 (8%)
Fluids (Peritoneal dialysis fluid, ascitic fluid)	7 (9.3%)
Devices (Catheter tip)	1 (1.3%)

Most of the *Acinetobacter baumannii* were isolated from respiratory specimens (29.3%; endotracheal aspirate and sputum) followed by pus (28%).

Fig:4 Sample wise distribution of *P.aeruginosa* and *A.baumannii*

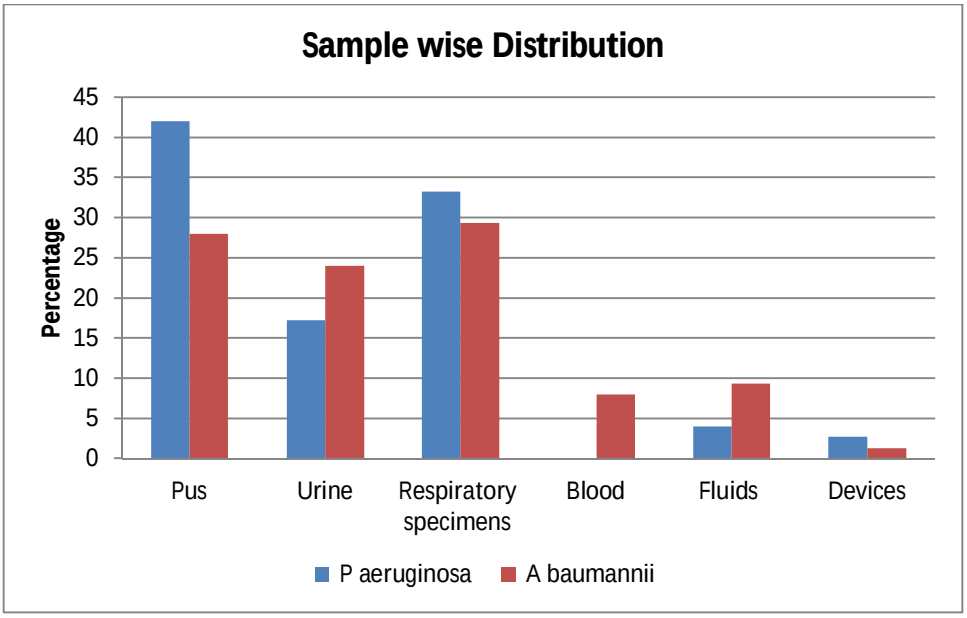


Table 13: Distribution of *Pseudomonas aeruginosa* isolates among various Clinical settings (n=75)

Clinical Specialty	No. of isolates (%)
ICU	7 (9.3%)
Surgical wards	28 (37.3%)
Medicine wards	16 (21.3%)
Renal Unit	12 (16%)
Orthopedics	12 (16%)

P.aeruginosa was isolated more commonly from the surgical wards which included general surgery, neurosurgery, vascular surgery, ENT ward and cardiothoracic surgery.

Fig 5: Distribution of *P.aeruginosa* among various clinical settings

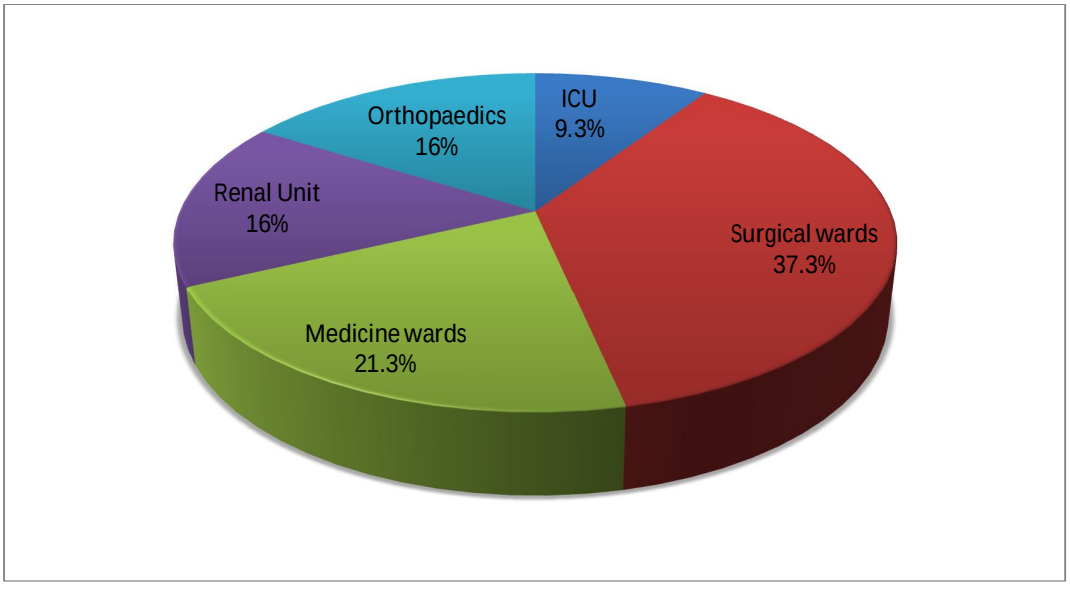
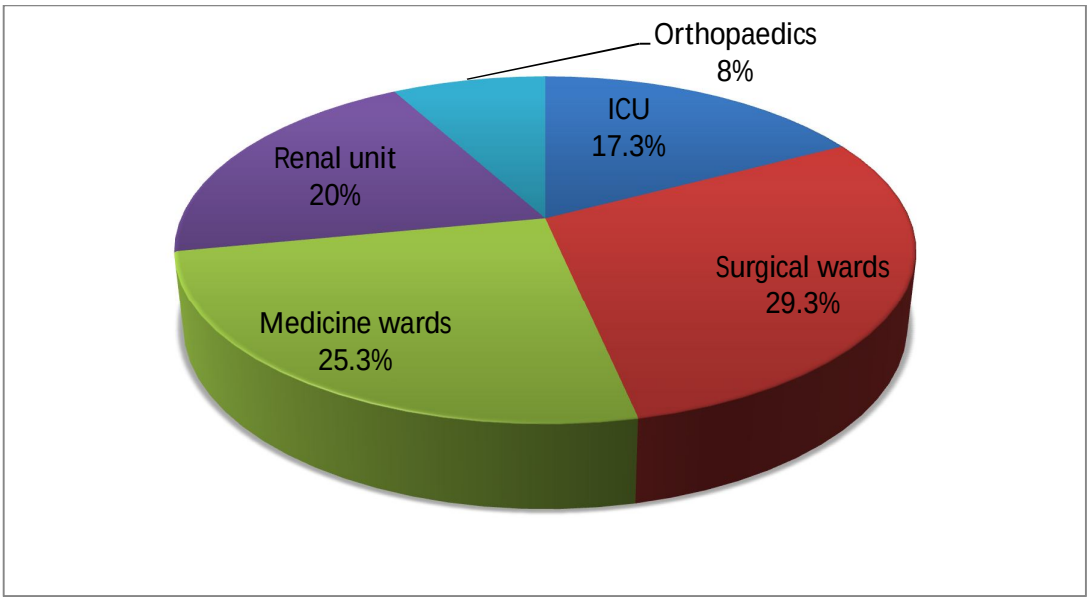


Table 14: Distribution of *Acinetobacter baumannii* isolates among various clinical settings (n=75)

Clinical Specialty	No. of isolates (%)
ICU	13 (17.3%)
Surgical wards	22 (29.3%)
Medical wards	19 (25.3%)
Renal unit	15 (20%)
Orthopedics	6 (8%)

Fig 6: Distribution of *A.baumannii* isolates among various clinical settings



A.baumannii isolates were predominantly isolated from surgical wards (29.3%) which included general surgery, vascular surgery, neurosurgery and cardiothoracic surgery.

Table 15: Antimicrobial Susceptibility pattern of *Pseudomonas aeruginosa* isolates (n=75)

Antimicrobial agent	No. of susceptible isolates (%)	No. of resistant isolates (%)
Ceftazidime (30 µg)	26 (34.7%)	49 (65.3%)
Piperacillin-Tazobactam (100/10µg)	52 (69.3%)	23 (30.7%)
Gentamicin (10 µg)	48 (64%)	27 (36%)
Cefepime (30 µg)	42 (56%)	33 (44%)
Amikacin (30 µg)	63 (84%)	12 (16%)
Ciprofloxacin (5 µg)	45 (60%)	30 (40%)
Meropenem (10 µg)	66 (88%)	9 (12%)
Imipenem (10 µg)	71 (94.7%)	4 (5.3%)

Higher susceptibility was seen with meropenem (88%) followed by amikacin (84%). Nine out of 75 isolates (12%) were resistant to meropenem whereas only four isolates were resistant to Imipenem (5.3%); these belong to MRIS (Meropenem Resistant Imipenem Sensitive) phenotype.

Table 16: Antimicrobial Susceptibility pattern of *Acinetobacter baumannii* isolates (n=75)

Antimicrobial agent	No. of susceptible isolates (%)	No. of resistant isolates (%)
Ceftazidime (30 µg)	14 (18.7%)	61 (81.3%)
Ciprofloxacin (5 µg)	35 (46.7%)	40 (53.3%)
Gentamicin (10 µg)	22 (29.3%)	53 (70.1%)
Meropenem (10 µg)	65 (86.7%)	10 (13.3%)
Imipenem (10 µg)	65 (86.7%)	10 (13.3%)
Piperacillin-Tazobactam (100/10µg)	48 (64%)	27 (36%)
Amikacin (30 µg)	51 (68%)	24 (32%)
Trimethoprim-sulfamethoxazole (1.25/23.75µg)	14 (18.7%)	61 (81.3%)
Tetracycline (30 µg)	42 (56%)	33 (44%)

A.baumannii isolates were highly susceptible to meropenem and imipenem (86.7%) whereas they are least susceptible to ceftazidime and trimethoprim-sulfamethoxazole (18.7%).

Table 17: Distribution of Multidrug Resistance and Carbapenem resistance among *P.aeruginosa* and *A.baumannii* isolates

Organism	No. of isolates		
	Total isolates (n)	MDR (%)	Carbapenem resistant (%)
Pseudomonas aeruginosa	75	22 (29.3%)	9 (12%)
Acinetobacter baumannii	75	38 (50.7%)	10 (13%)

MDR – Multidrug resistant

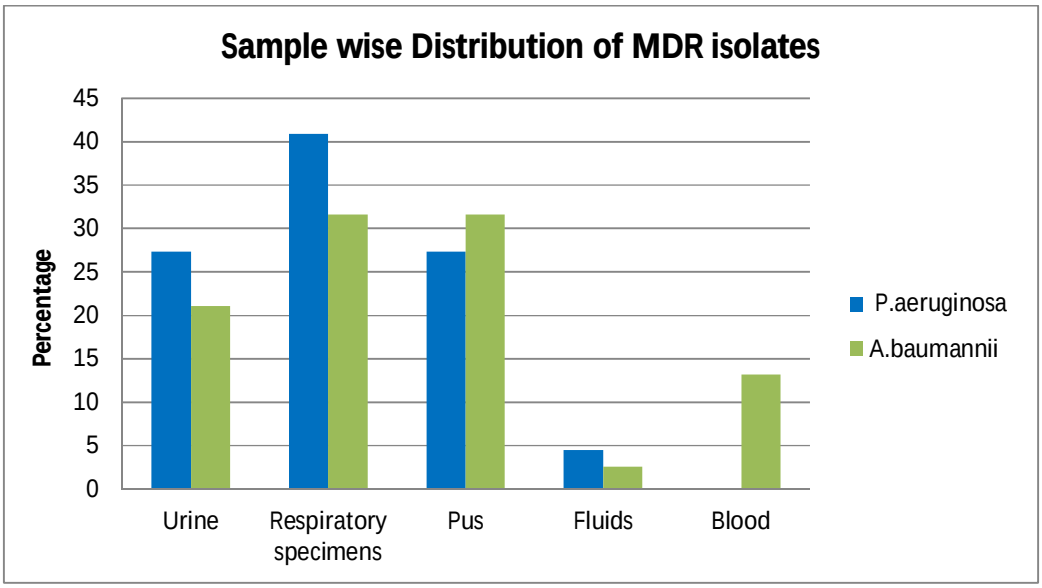
Among 75 *P.aeruginosa* isolates, about 29.3% and 12% were multidrug resistant and carbapenem resistant, respectively; among *A.baumannii* isolates, 50.7% were multidrug resistant whereas 13% were Carbapenem Resistant *A.baumannii* (CRAB).

Table 18: Distribution of Multidrug Resistant *P.aeruginosa* and *A.baumannii* isolates among various samples

Clinical samples	No. of MDR *P.aeruginosa* isolates (n=22)	No. of MDR *A.baumannii* isolates (n=38)
Urine	6 (27.3%)	8 (21.1%)
Respiratory specimens	9 (40.9%)	12 (31.6%)
Pus	6 (27.3%)	12 (31.6%)
Fluids	1 (4.5%)	1 (2.6%)
Blood	-	5 (13.2%)

MDR – Multidrug resistant

Fig: 7 Sample wise distribution of MDR isolates



MDR *P.aeruginosa* isolates were predominantly from respiratory specimens (which included endotracheal aspirate - 22.7%, sputum – 13.6%, bronchial wash – 4.5%) whereas multidrug resistant *A.baumannii* isolates were equally distributed between respiratory specimens (which included endotracheal aspirate and sputum) and pus samples (31.6)%.

Table 19: Phenotypic characterization of resistance among *Pseudomonas aeruginosa* isolates (n=75)

Phenotypic tests	No. of isolates positive (%)
ESBL detection	17 (22.7%)
AmpC detection	11 (14.7%)
ESBL & AmpC	-
Carbapenem resistant (by disc diffusion)	9 (12%)
Carbapenemase production by Modified Hodge test	4 (5.3%)
MBL detection	4(5.3%)
AmpC & MBL	-

From this table, ESBL was the most common resistance mechanism against β-lactams for *P.aeruginosa*. Among the carbapenem resistant isolates (n=9), only four isolates were resistant to both imipenem and meropenem; these belong to IRMR phenotype which is predominantly enzyme (metallo-β-lactamase) mediated; other five isolates were MRIS phenotype which is mainly due to efflux pump. Therefore, the carbapenem resistance in *P.aeruginosa* is predominantly non-enzyme mediated.

Table 20: Phenotypic characterization of resistance among *Acinetobacter baumannii* isolates (n=75)

Phenotypic tests	No. of isolates positive (%)
ESBL detection	27 (36%)
AmpC detection	19 (25.3%)
ESBL & AmpC	2 (2.7%)
Carbapenem resistant (by disc diffusion)	10 (13.3%)
Carbapenemase production by Modified Hodge test	9 (12%)
MBL detection	3 (4%)
AmpC & MBL	1 (1.3%)
Carbapenemase & AmpC co-production	2 (2.7%)

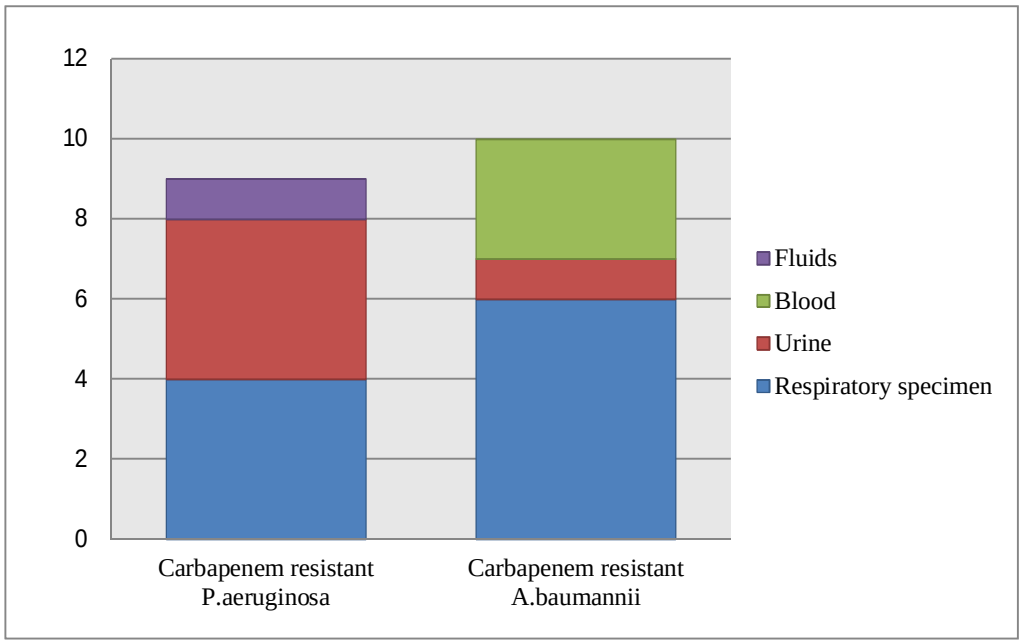
Among *A.baumannii*, ESBL was the predominant resistance mechanism against β-lactams. All the 10 carbapenem resistant isolates were resistant to both imipenem and meropenem which belong to IRMR phenotype (enzyme mediated); only three of them were found to be MBL producers. This indicates that carbapenem hydrolyzing enzyme production was the predominant resistance mechanism against carbapenem in *A.baumannii.*

Table 21: Distribution of Carbapenem resistant *P.aeruginosa* and *A.baumannii* isolates among various samples

Clinical samples	No. of Carbapenem resistant *P.aeruginosa* (n=9)	No. of Carbapenem resistant *A.baumannii* (n=10)
Respiratory specimens	4 (44.4%)	6 (60%)
Urine	4 (44.4%)	1 (10%)
Blood	-	3 (30%)
Fluids	1 (11.1%)	-
***p value**	**0.006**	**0.007**

*p < 0.05 – Statistically significant

Fig 8: Distribution of Carbapenem resistant *P.aeruginosa* and *A.baumannii* isolates among clinical samples



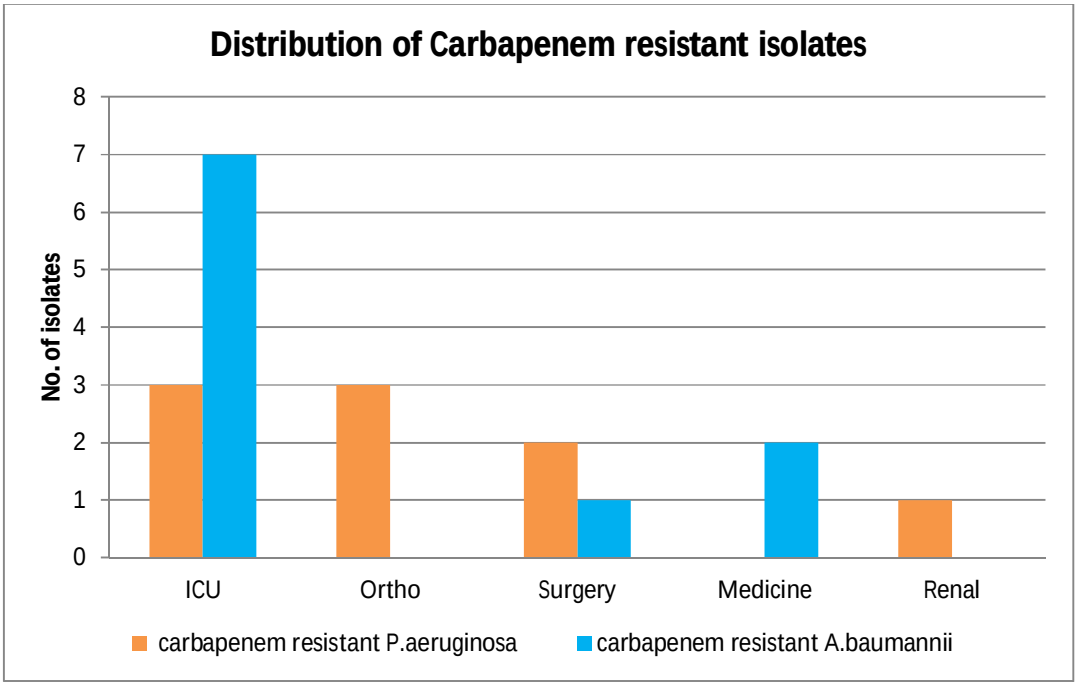
Carbapenem resistant *P.aeruginosa* and *A.baumannii* were predominantly isolated from respiratory specimens (especially from endotracheal aspirate). This association was found to be statistically significant.

Table 22: Distribution of Carbapenem resistant isolates among various Clinical settings (n=19)

Clinical Wards	No. of carbapenem resistant *P.aeruginosa* (n=9)	No. of carbapenem resistant *A.baumannii* (n=10)	Total (n=19)
ICU	3	7	10 (52.6%)
Orthopaedic ward	3	-	3 (15.8%)
Surgical wards	2	1	3 (15.8%)
Medicine wards	-	2	2 (10.5%)
Renal Unit	1	-	1 (5.3%)
***p value**	0.047	0.001	19

*p < 0.05 – significant

Fig: 9 Distribution of Carbapenem resistant isolates



Majority of the carbapenem resistant *P.aeruginosa* and *A.baumannii* isolates were found to be from Intensive care unit (52.6%) which is statistically significant ($p < 0.05$).

Table 23: Minimum Inhibitory Concentration of Imipenem for the Imipenem resistant isolates by disc diffusion method (n=14)

Organism	No. of isolates with Minimum Inhibitory Concentration (MIC) - µg/ml		
	≤2 Sensitive	4-8 Intermediate	≥8 Resistant
P.aeruginosa (n=4)	-	1	3
A.baumannii (n=10)	-	2	8

Among the 14 Imipenem resistant isolates, three isolates (one *P.aeruginosa* and two *A.baumannii* isolates) were with MIC in the intermediate range.

Table 24: Risk factors associated with carbapenem resistance (n=19)

Risk factors	Occurrence (%)
Hospital stay > 7 days	14 (73.7%)
Mechanical Ventilation	12 (63.2%)
ICU admission	10 (52.6%)
Urinary catheterization	18 (94.7%)
Previous use of antibiotic (Carbapenem)	5 (26.3%)
Previous surgery	3 (15.8%)

***Mortality – 6 (31.2%)**

Carbapenem resistance rate was high in the ICU due to these risk factors such as prolonged hospital stay, interventions (mechanical ventilation, catheterization) and previous use of antibiotic especially carbapenems.

Table 25: Antimicrobial resistance pattern among Carbapenem susceptible and Carbapenem resistant *P.aeruginosa* isolates

Antibiotic	No. (%) of resistant isolates among			
	Total (n=75)	**Carbapenem susceptible isolates (n=66)**	**Carbapenem resistant isolates (n=9)**	***p value**
Ceftazidime (30µg)	49 (65.3%)	40 (60.6%)	9 (100%)	0.023
Cefepime (30 µg)	33 (44%)	24 (36.4%)	9 (100%)	0.0001
Piperacillin-Tazobactam (100/10µg)	23 (30.7%)	14 (21.2%)	9 (100%)	0.0001
Amikacin (30 µg)	12 (16%)	8 (12.1%)	4 (44.4%)	0.032
Gentamicin (10µg)	27 (36%)	19 (28.8%)	8 (88.8%)	0.001
Ciprofloxacin (5µg)	30 (40%)	22 (33.3%)	8 (88.8%)	0.002

*$p < 0.05$ – Statistically significant

Table 26: Antimicrobial resistance pattern among Carbapenem susceptible and Carbapenem resistant *A.baumannii* isolates

Antibiotic	No. (%) of resistant isolates among			
	Total (n=75)	**Carbapenem susceptible isolates (n=65)**	**Carbapenem resistant isolates (n=10)**	***p value**
Ceftazidime (30µg)	61 (81.3%)	51 (78.5%)	10 (100%)	0.109
Piperacillin-Tazobactam (100/10µg)	27 (36%)	17 (26.2%)	10 (100%)	0.0001
Amikacin (30 µg)	24 (32%)	16 (24.6%)	8 (80%)	0.001
Gentamicin (10µg)	53 (70.1%)	43 (66.2%)	10 (100%)	0.024
Ciprofloxacin (5µg)	40 (53.3%)	32 (49.2%)	8 (80%	0.068
Trimethoprim-Sulfamethoxazole (1.25/23.75 µg)	61 (81.3%)	51 (78.5%)	10 (100%)	0.109
Tetracycline (30µg)	33 (44%)	25 (38.5%)	8 (80%)	0.09

*p< 0.05 – Statistically significant

Isolates which were found to be carbapenem resistant were also resistant to many of the other antibiotics and this was statistically significant.

Table 27: Molecular characterization of MBL positive *P.aeruginosa* isolates (n=4)

MBL positive *P.aeruginosa* (n=4)	bla_{VIM} positive	bla_{NDM-1} positive	bla_{VIM} & bla_{NDM-1} negative
	1	2	1

Genotypic characterization among the four MBL producing *P.aeruginosa*, two were found to be bla_{NDM-1} positive and one bla_{VIM} positive.

Table 28: Molecular characterization of MHT positive *A.baumannii* isolates (n=9)

Carbapenemase producing (MHT positive) *A.baumannii* (n=9)	bla_{OXA-23} positive	bla_{OXA-23} negative
	4	5

Genotypic characterization among the nine carbapenemase producing *A.baumannii*, four were found to be bla_{OXA-23} positive.

Table 29: MIC of Colistin for the Carbapenem resistant isolates (n=19)

Organism	No. of isolates with Minimum Inhibitory Concentration (MIC) - µg/ml			
	Sensitive			Resistant
	≤ 0.5	0.5 – 1	1 – 2	≥ 4
P.aeruginosa (n=9)	2	4	3	-
A.baumannii (n=10)	5	4	1	-

All the carbapenem resistant *P.aeruginosa* and *A.baumannii* were susceptible to colistin by E-test. Four isolates (three strains of *P.aeruginosa* and one strain of *A.baumannii*) were having MIC in the upper limit of susceptible range (1.5 to 2 µg/ml).

Discussion

DISCUSSION

Currently, antimicrobial resistance especially for carbapenem is a major public health issue which increases morbidity and mortality in hospitalized patients. Among the various mechanisms for carbapenem resistance, carbapenemase production is plasmid mediated; these resistance genes on plasmids can be transferred horizontally from one bacterium to another bacterial species. This is the major cause of dissemination of antimicrobial resistance genes between various bacterial species. Therefore, identifying the prevalence and mechanism of carbapenem resistance and their susceptibility to other antibiotics are necessary to formulate antibiotic policies in a hospital set–up and to determine various treatment options.

In this study, the majority of *Pseudomonas aeruginosa* isolates were from pus sample (42.7%), followed by respiratory specimens (33.3%). Similarly, *Acinetobacter baumannii* isolates were predominantly isolated from respiratory specimens (29.3%) followed by pus sample (28%) – Table 11&12. In a study conducted by *Shasikala et al* at Pondicherry, 27.6% of the *P.aeruginosa* isolates were from wound infections. *Padmalakshmi et al* reported 37.5% of *A.baumannii* isolates were from respiratory specimen which is similar to this study. [7,60] Both *P.aeruginosa* and *A.baumannii* are ubiquitous, can tolerate harsh environments and hence, colonizes the skin and respiratory tract more commonly in hospitalized patients.

Regarding specialty wise distribution, *P.aeruginosa* was predominantly isolated from surgical units (37.3%) mainly from post operative infections and patients with diabetic foot; followed by medical wards (21.3%); similarly, *A.baumannii* was isolated mostly from surgical wards (29.3%), followed by medicine wards (25.3%) – Table 13&14.

The overall resistance of *P.aeruginosa* vs *A.baumannii* to the antibiotics tested was ceftazidime (65.3% vs 81.3%), pipercillin-tazobactam (30.7% vs 36%), amikacin (16% vs 32%), gentamicin (36% vs 70.1%), ciprofloxacin (40% vs 53.3%), meropenem (12% vs 13.3%) and imipenem (5.3% vs 13.3%) – Table 15&16.

This correlates with a study from United States, in which among *Acinetobacter* species carbapenem resistance rates have been reported from 34% to as high as 62.6% and National Healthcare Safety Network (NHSN) in the United States have reported an increase from 33% carbapenem resistance in 2006 to 2007 to >60% among *Acinetobacter* species isolates in 2009 to 2010.[61,62,63] According to the study by *Hong D J et al,* carbapenem resistance rates among *Pseudomonas aeruginosa* isolates in most countries range from 10% to 50% and have been reported as low as 3.3% in Canada to >50% in Russia, Southwest Asia, and South America.[64]

Mohanty et al in their study at New Delhi found that the overall resistance of the isolates (*P. aeruginosa* vs *Acinetobacter* spp.) to the antibiotics was ceftazidime (57.9% vs 84.0%), piperacillin/tazobactam (22.1% vs 42.0%),

amikacin (33.7% vs 72.0%), gentamicin (40.0% vs 80.0%), ciprofloxacin (35.8% vs 64.0%), meropenem (36.8% vs 62.0%) and imipenem (37.9% vs 64.0%).[4] *Benacchinmardi et al* in a recent study at PGIMSR, Bengaluru showed 80% of *P.aeruginosa* and about 41% *A.baumannii* were sensitive to imipenem.[65] According to the study conducted at Trichy in 2015, 8.7% of NFGNB were resistant to meropenem.[66] This is concordant with the present study which shows about 12% carbapenem resistance in *P.aeruginosa* and 13.3% in *A.baumannii*. These differences in the antimicrobial susceptibility could be due to the geographical variation. Therefore, various international authorities emphasize that every hospital should have its own antibiotic policy.

In this study, the prevalence of MDR among *P.aeruginosa* was found to be 29.3% and among *A.baumannii* was 50.7% (Table 17). This is similar to the study conducted in Italy by *Franesco De et al* who showed the prevalence of MDR among *P.aeruginosa* and *A.baumannii* as 20% and 54% respectively.[67] *Khan F et al* reported 30% of *P.aeruginosa* isolates as MDR[68], while *Lakshmi et al* found 77% of *A.baumannii* to be multidrug resistant.[69]

Sample wise MDR *P.aeruginosa* was isolated predominantly from respiratory tract specimens (endotracheal aspirate, sputum and bronchial wash) accounting for 40.9%; MDR *A.baumannii* was equally distributed between respiratory tract specimens and pus (31.6%) – Table 18. This is in correlation with most of the studies[67, 68, 69]where MDR *P.aeruginosa* and *A.baumannii* were predominantly isolated from specimens of the respiratory tract.

Among *P.aeruginosa*, 22.7% strains were phenotypically characterized as ESBL producers, 14.7% were AmpC producers, 5.3% were positive for carbapenemase production by Modified Hodge test and all these were found to be Metalloβ-lactamase by Imipenem-EDTA combined disc method; while among *A.baumannii* 36% strains were ESBL producers, 25.3% were AmpC producers, 2.7% were Co-producers of ESBL and AmpC, 12% were positive for carbapenemase production by Modified Hodge test, 4% were metalloβ-lactamase producers and 4% were co-producers of carbapenemase and AmpC (Table 19&20) .

According to the study of *Gupta et al,* among non-fermenters 21.4% were ESBL producers, 51.1% were AmpC producers and 21.4% were metallobeta-lactamase producers.[70] Since in this study sample size is low and being a single centered study, the true prevalence of resistance mechanisms could not be completely evaluated. Multi-centered study with large sample size is required to find the overall prevalence of resistance mechanisms in the community.

Five of the nine meropenem resistant *P.aeruginosa* isolates were sensitive to Imipenem; they were categorized as MRIS (Meropenem Resistant Imipenem Sensitive). In MRIS phenotype, the carbapenem resistance could be due to over expression of efflux pump which can be confirmed by the genotypic methods. In *A.baumannii*, all the carbapenem resistant isolates were resistant to both meropenem and imipenem; they belong to IRMR (Imipenem Resistant Meropenem Resistant) phenotype in which the carbapenem resistance is

predominantly enzyme mediated (carbapenem hydrolyzing enzymes such as OXA type carbapenemases and Ambler class B metalloβlactamases).[71]

The emergence of these phenotypes occurs mainly due to the antibiotic selection pressures promoted by inappropriate dosage and duration of the carbapenems. Hence, it is advisable to perform antimicrobial susceptibility testing for each of the carbapenems namely imipenem, meropenem and doripenem, rather than testing single carbapenem and to extrapolate the results for other carbapenems.

In *P.aeruginosa*, carbapenem resistant isolates were equally distributed between respiratory specimen especially endotracheal aspirate and urine (44.4%) whereas in *A.baumannii*, carbapenem resistance was noted predominantly among respiratory specimen (endotracheal aspirate - 50% & sputum 10%) as most of these isolates were from ICU patients on mechanical ventilation (Table 21&22).

Of the total 19 carbapenem resistant isolates (nine *P.aeruginosa* and ten *A.baumannii*), 52.6% were isolated from patients admitted in Intensive care units. This correlates with the studies conducted in Iran and New Delhi which reported 53.6% and 67.5% carbapenem resistance among patients admitted in intensive care unit respectively.[72, 73] This high carbapenem resistance rate in ICU is due to the associated risk factors such as prolonged hospital stay, interventions such as mechanical ventilation and previous use of antibiotics especially carbapenem.

This increase in carbapenem resistance in ICU is alarming and hence it is necessary to take various preventive measures which include screening for carbapenem resistance carriers in high risk units (surveillance cultures), undertaking strict contact precautions for carriers and antibiotic stewardship programs to spare carbapenems.

Among the 14 imipenem resistant isolates (four *P.aeruginosa* and ten *A.baumannii*), three isolates showed MIC of imipenem in the intermediate range (Table 23). Recent studies showed that extended infusion therapy of carbapenem for about 30 minutes to 3 hours was found to be effective if the MIC of carbapenem falls in the intermediate range (4µg/ml to <8µg/ml).[26, 46, 47] Hence, mere screening for carbapenem susceptibility is insufficient and detection of MIC is essential as it can determine the appropriate treatment regimens.

Carbapenem resistant *P.aeruginosa* isolates were also 100% resistant to ceftazidime, cefepime and piperacillin-tazobactam; however they were highly sensitive to amikacin (55.6%). Similarly, carbapenem resistant *A.baumannii* isolates were 100% resistant to ceftazidime, piperacillin-tazobactam, gentamicin and trimethoprim-sulfamethoxazole (Table 25&26). This is statistically significant ($p<0.05$) and in parallel to the study by *Mohanty et al*, which showed carbapenem resistant *P.aeruginosa* and *A.baumannii* were significantly ($p< 0.05$) resistant to other antibiotics.[4]

Since carbapenem resistance is predominantly mediated by multi-drug resistance transferrable plasmids, carbapenem resistant strains remain resistant to

several other antibiotics including fluoroquinolones, aminoglycosides, third generation cephalosporins such as ceftazidime and β-lactam/β-lactamase inhibitor combinations. This poses serious problems in choosing the right antibiotic for the treatment of hospitalized patients admitted in ICU. This can be controlled by various strategies such as strict infection control measures, judicious use of antibiotics, antibiotic resistance surveillance programs and antibiotic cycling.

Out of four MBL producing *P.aeruginosa*, two isolates were positive for $bla_{\text{NDM-1}}$ gene by conventional PCR, one isolate was positive for bla_{VIM} gene and one was negative for both; five carbapenem resistant *P.aeruginosa* isolates were MRIS phenotypes which could be due to over expression of efflux pumps. Of the nine carbapenemase producing *A.baumannii*, four were positive for $bla_{\text{OXA-23}}$ gene and three were found to be metalloβ-lactamase producers by phenotypic method (Table 27&28). Therefore the predominant mechanism for carbapenem resistance among *P.aeruginosa* and *A.baumannii* was efflux pump over expression and carbapenemase production respectively.

This correlates well with the study by *Gniadek T J et al* which states that "Currently, in the United States and Europe, OXA type carbapenemase production (predominantly OXA-23) is the primary resistance mechanism among *A. baumannii*, while the loss of OprD porin expression, without the expression of a carbapenemase, is the primary mechanism of resistance among *P.aeruginosa*".[58,74] In a study at Christian Medical College, Vellore, IRMS (due to loss of outer membrane protein) and MRIS phenotypes of *P. aeruginosa* were more commonly

observed rather than IRMR phenotype which is due to the plasmid mediated carbapenemases.[71]

All the 19 carbapenem resistant isolates were 100% susceptible to colistin. *Baurah FK et al* in a study in 2014 reported that *P.aeruginosa* was 100% susceptible to Colistin.[54] However according to *Mohanty et al,* in India, the prevalence of colistin resistance was found to be 6% in *A.baumannii* and about 8% in *P.aeruginosa* which is in contrast to this study. *Taneja et al* also reported about 3.5 % of *A.baumannii* to be colistin resistant.[4, 53] This variation in the prevalence of colistin resistance could be due to the geographic variation and different antibiotic policies among various hospitals.

Four of the 19 carbapenem resistant isolates were having MIC in the upper limit of susceptible range (1.5 to 2 µg/ml) – Table 29; this indicates that the MIC testing for colistin should be made mandatory before administration to prevent the emergence of colistin resistance in a community, as colistin is the only available effective antibiotic for the treatment of carbapenem resistant infections.

Despite the risk of nephrotoxicity in patients receiving colistin, colistin may be useful for salvage therapy of carbapenem resistant *P. aeruginosa* and *A.baumannii* infections where the therapeutic choices are severely limited.

Limitations of the study

LIMITATIONS OF THE STUDY

Since this is a single centered study with low sample size, the true prevalence of resistance mechanisms could not be evaluated. Multi-centered studies with larger sample size are required to identify the overall prevalence of resistance mechanisms in the community.

Molecular characterization for the carbapenem resistant isolates was done only for the most prevalent genes (*bla*$_{NDM-1}$, *bla*$_{VIM}$and *bla*$_{OXA-23}$). There are various other genes responsible for carbapenem resistance, which were not included in this study.

Summary

SUMMARY

- The prevalence of antibiotic susceptibility and resistance mechanisms of 75 *Pseudomonas aeruginosa* and 75 *Acinetobacter baumannii* isolates from various clinical specimens were studied.
- This study shows 29.3% of *P.aeruginosa* and 50.7% *A.baumannii* as MDR; of this 12% of *P.aeruginosa* and 13.3% of *A.baumannii* were carbapenem resistant.
- The carbapenem resistant isolates were predominantly from respiratory specimen especially endotracheal aspirate; their most common source was intensive care unit patients.
- Efflux pump over expression followed by metalloβ-lactamase was the predominant mechanism for carbapenem resistance in *P.aeruginosa* while in *A.baumannii*, carbapenemase (oxacillinase type) production was the predominant mechanism.
- Among the four MBL positive *P.aeruginosa* isolates, two isolates were positive for bla_{NDM-1}gene and one isolate was positive for bla_{VIM} gene.
- Among the Carbapenemase producing *A.baumannii*, four isolates were positive for bla_{OXA-23} gene.
- Carbapenem resistant isolates were also resistant to many other antibiotics but 100% susceptible to colistin.
- Colistin MIC of four carbapenem resistant isolates was in the upper limit of susceptible range.

Conclusion

CONCLUSION

Carbapenem resistance is increasing in the post-antibiotic era under the selection pressure of carbapenem in clinical settings. Detection of carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* has great impact on hospital infection control and for epidemiological purpose for the prevention of further spread of resistance in the community. To overcome the resistance, implementation of strict infection control practices and active surveillance of genes encoding carbapenemase are necessary.

Because carbapenem resistance is also associated with resistance to antibiotics of other classes, the therapeutic options are very limited. Although colistin may be considered as an alternative for infections caused by carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, MIC testing should be performed whenever clinical use of colistin is considered to contain the emerging colistin resistance.

Colour plates

COLOUR PLATES

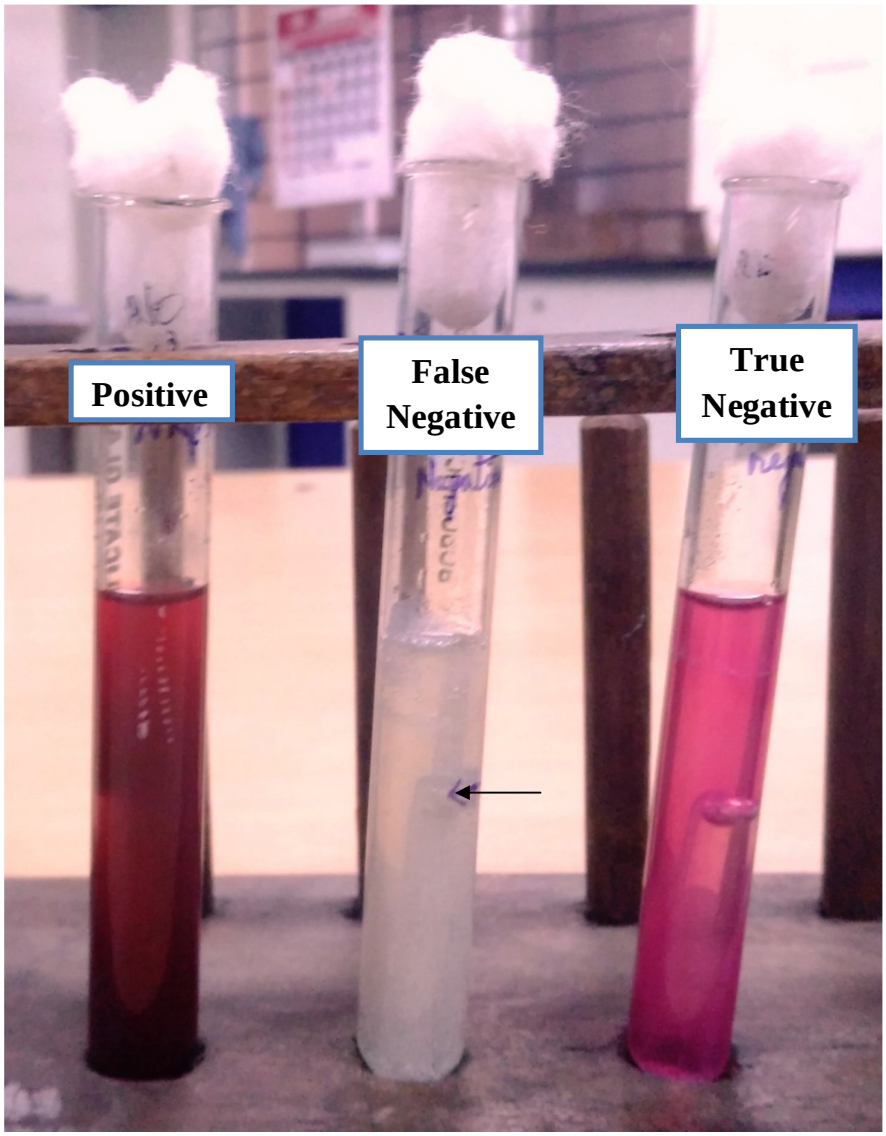


Fig:10 Nitrate reduction test

Positive control – Red colour eg. *Escherichia coli*

False Negative – No colour change even after addition of Zinc dust;
Arrow shows gas production. Eg.*Pseudomonas aeruginosa*

True Negative – Pink colour develops after adding zinc dust;
eg. *Acinetobacter baumannii*

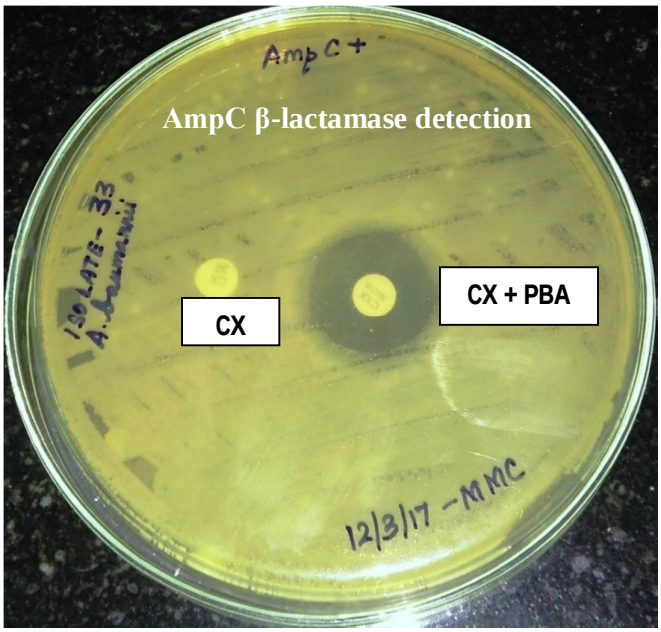


Fig : 11 AmpC betalactamase detection by combined disc method using cefoxitin (30µg) and cefoxitin + Phenylboronic acid (PBA) – 30/400 µg. Zone of inhibition around the inhibitor combination is ≥5mm than around the cefoxitin disc alone.

Fig:12 MRIS – Meropenem Resistant Imipenem Sensitive phenotype of Carbapenem resistance in *Pseudomonas aeruginosa*

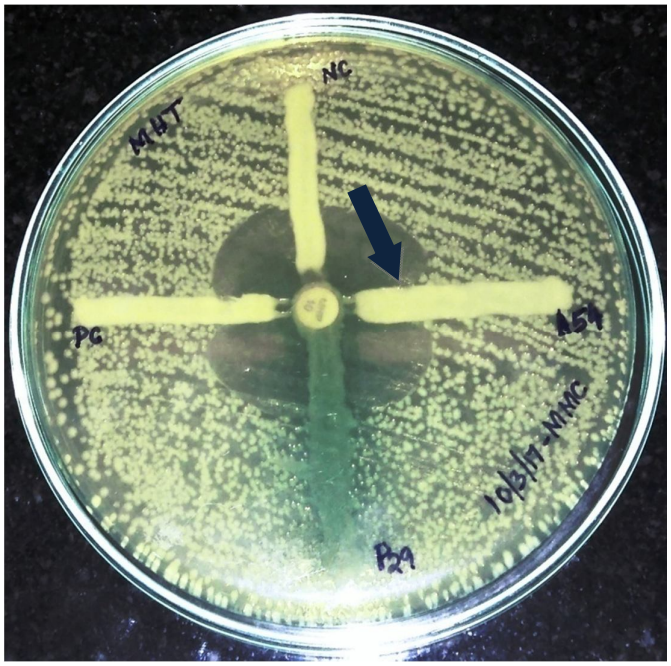


Fig:13Modified Hodge Test (MHT)

A 54 & P 29 – MHT positive for carbapenemase production.

Arrow shows Clover-leaf indentation

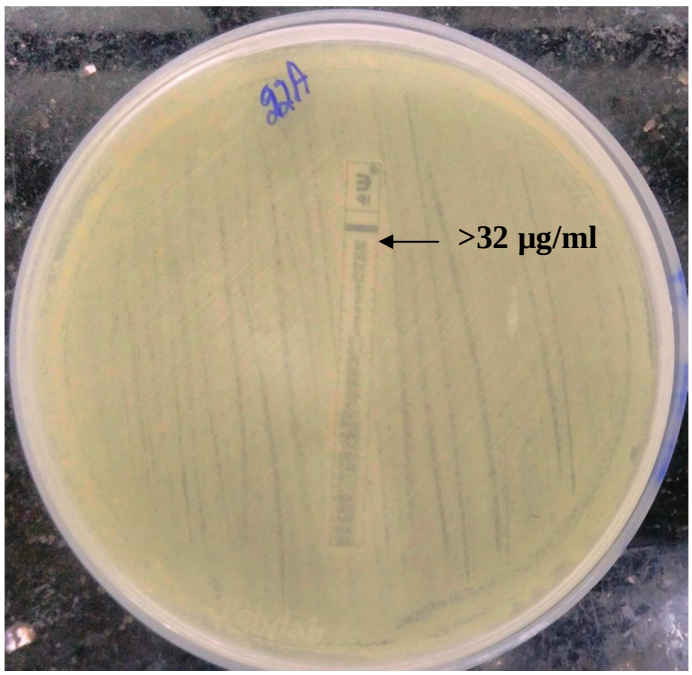


Fig:14 Imipenem MIC detection by E-test showing MIC >32µg/ml (Carbapenem resistant *A.baumannii* – CRAB isolate)

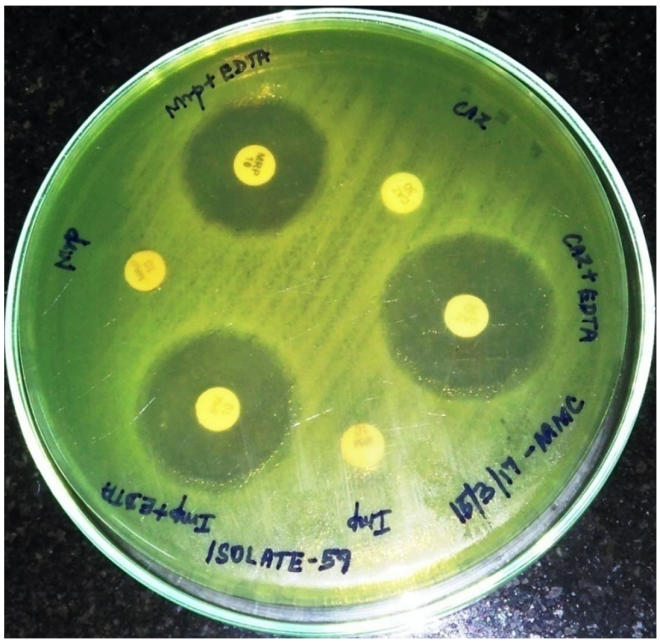


Fig:15 Metallo Beta-Lactamase (MBL) detection by Combined disc method using Imipenem with Imipenem-EDTA (10/750µg/ml), Meropenem with Meropenem-EDTA (10/930µg/ml) & Ceftazidime with Ceftazidime-EDTA (10/930µg/ml)

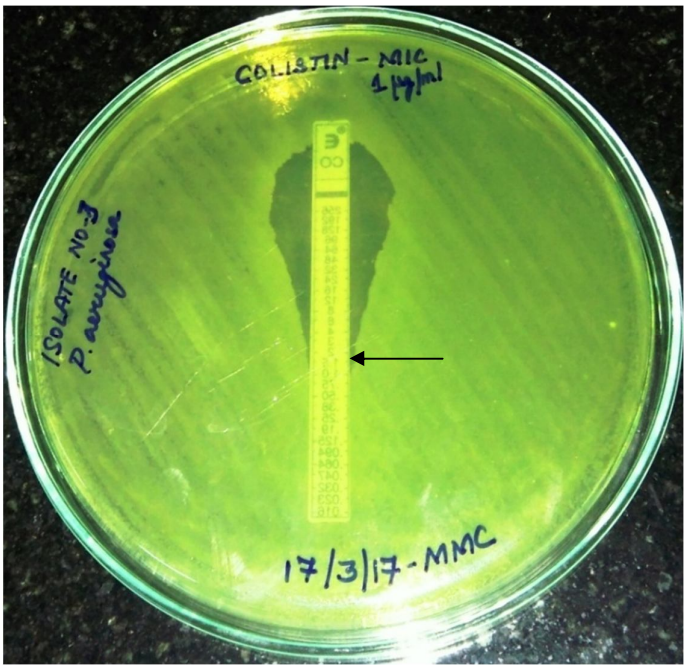


Fig:16 Colistin MIC by E-test for Carbapenem resistant *Pseudomonas aeruginosa* showing MIC of 1µg/ml (colistin sensitive)

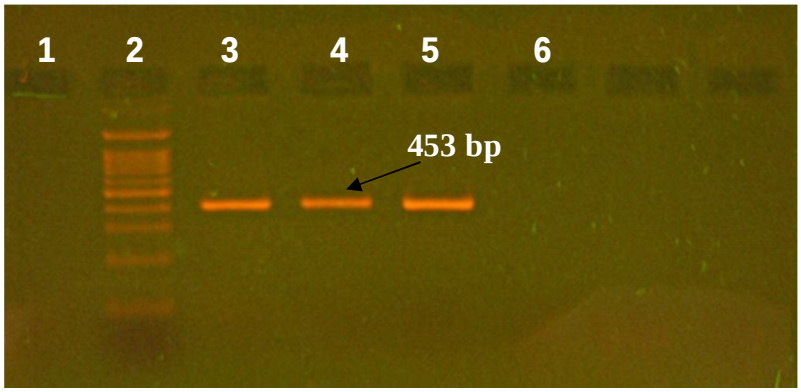


Fig:17 Polymerase Chain Reaction (PCR) for the detection of bla_{OXA-23} gene in Carbapenemase producing *Acinetobacter baumannii*

Lane 1- Non-template Control Lane2- DNALadder

Lane 3,4,5 – *A.baumannii* isolates positive for bla_{OXA-23} gene

Lane 6- Negative for bla_{OXA-23} gene

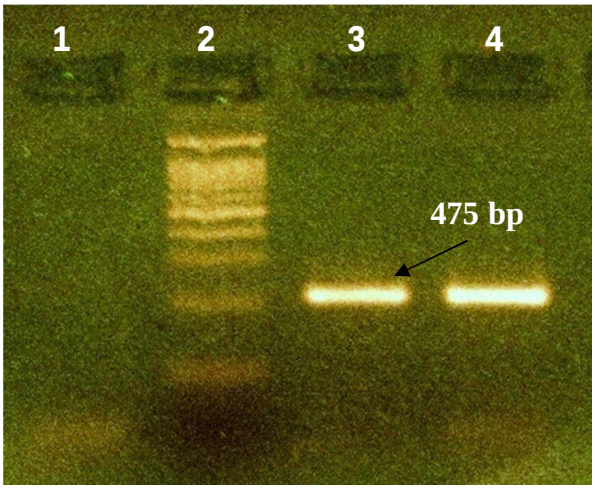


Fig:18 Conventional PCR for the detection of bla_{NDM-1} gene in Metallo Beta Lactamase (MBL) producing *Pseudomonas aeruginosa*

Lane 1- Non-template Control Lane 2- DNALadder

Lane3,4 – *P.aeruginosa* isolates positive for bla_{NDM-1} gene

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Annexures

ANNEXURES

PROFORMA

- Name : IP NO:
- Age: Ward:
- Sex:
- Occupation:
- Address:
- Presenting complaints
- Past history
- Prior antibiotic therapy
- Clinical Diagnosis
- Microbiological investigation:
  - Direct examination :
- Bacterial Culture :
- Speciation
- Antibiotic sensitivity pattern -
- PCR for Carbapenemase genes –

CONSENT FORM

STUDY TITLE : Characterization and Colistin susceptibility of Carbapenem resistant isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in a tertiary care hospital

I ……, hereby give consent to participate in the study conducted by Dr.M.Sowndarya, Post graduate at Institute of Microbiology, Madras Medical College, Chennai and to use my personal clinical data and the result of investigations for the purpose of analysis and to study the nature of the disease, I also give consent to give my clinical Specimen (sputum, endotracheal aspirate, bronchial wash, pleural fluid, ascetic fluid, peritoneal dialysis fluid, wound swab, cerebrospinal fluid, urine, pus, blood) for further investigations. I also learn that there is no additional risk in this study. I also give my consent for my investigator to publish the data in any forum or journal.

Signature/ Thumb impression Of the patient/ relative

Place

Date

Patient Name & Address:

Signature of the investigator:

Signature of guide:

INFORMATION SHEET

STUDY TITLE : Characterization and Colistin susceptibility of Carbapenem resistant isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in a tertiary care hospital

INVESTIGATOR : **Dr.M.Sowndarya,**
Post Graduate,
Institute of Microbiology,
Madras Medical College,
Chennai - 600003

GUIDE : **Dr. ThasneemBanu S M.D.,**
Professor,
Institute of Microbiology,
Madras Medical College,
Chennai - 600003

In recent times, infection caused by multidrug resistant organisms has become a great threat. Multidrug resistance is more common in non-fermenter gram-negative bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Carbapenems are the drug of choice for cephalosporin resistant gram-negative bacterial infections. Carbapenem resistance is now an emerging threat. Therapeutic options for infections with these isolates include colistin.

I am going to detect the prevalence of carbapenem resistance among the isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* and their colistin susceptibility in this tertiary hospital. I am going to collect clinical specimens such as sputum, endotracheal aspirate, pleural fluid, bronchial wash, urine, blood and pus and process them accordingly. 150 patients are included in this study after getting informed consent. This study is entirely voluntary and patient can withdraw any time from this study. Extra cost will not be incurred to the patients. Any doubt regarding this study will be willingly clarified. Results of the study will be published. In case of any doubt please contact Dr.M.Sowndarya, Contact No:9442602762.

MASTER CHART - PSEUDOMONAS AERUGINOSA

S. No	Name	Age	Sex	IP.No	Ward	Diagnosis	Sample	AMK	GM	CIP	CAZ	Cefepime	PT	IMP	IMP MIC	MRP	Colistin	Resistance pattern	*bla* $_{NDM-1}$ */bla* $_{VIM}$
1	Selva	58	M	51229	Uro	UTI	Urine	S	S	R	R	S	S	S	-	S	-	ESBL	-
2	Muniyappan	56	M	49416	RTU	UTI	Urine	R	R	R	R	R	R	S	-	R	S	MDR/MRIS	-
3	Shobana	41	F	53551	Neuro	R Hemipleg	Urine	R	R	R	R	R	R	S	-	R	S	MDR/MRIS	-
4	Masthan	35	M	55166	RTU	UTI	Urine	S	S	S	R	S	S	S	-	S	-	ESBL	-
5	Manikandan	25	M	53465	Ortho	# R Femur	Urine	S	R	R	R	R	R	R	R	R	S	MDR / MBL	bla_{NDM-1}
6	Bavani	42	F	55096	IMCU	UTI	Urine	S	S	R	R	R	S	S	-	S	-	ESBL	-
7	Dhandapani	45	M	51972	Nephro	UTI	Urine	S	S	S	S	S	S	S	-	S	-	-	-
8	Indira	45	F	50043	Uro	UTI	Urine	R	R	R	R	S	S	S	-	S	-	MDR	-
9	Gunasundari	57	F	50981	Uro	UTI	Urine	S	R	S	S	S	S	S	-	S	-	-	-
10	Devasagayam	45	M	51021	Nephro	UTI	Urine	S	S	S	S	S	S	S	-	S	-	-	-
11	Samynathan	37	M	54625	TM	Bronchiectasis	Sputum	S	R	S	R	R	R	S	-	S	-	-	-
12	Anandhan	46	M	53389	TM	old PTB	Bronchial wash	S	S	S	R	S	S	S	-	S	-	-	-
13	Padmini	45	F	51299	TM	Bronchiectasis	Sputum	S	R	R	R	R	R	S	-	S	-	MDR/AmpC	-
14	Arumugam	40	M	51486	Ortho	RTA	Pus	S	S	S	S	S	S	S	-	S	-	-	-
15	Murugan	63	M	48846	SUR	DFS	Pus	S	S	R	R	R	S	S	-	S	-	ESBL	-
16	Kalidas	45	M	52132	Ortho	RTA	Pus	S	R	S	S	S	S	S	-	S	-	-	-
17	Bharani	35	M	52118	Ortho	BB Fracture	Pus	S	S	S	S	S	S	S	-	S	-	-	-
18	Shanmugam	46	M	53553	SUR	Liver abscess	Pus	S	S	S	R	S	S	S	-	S	-	ESBL	-
19	Subravelu	86	M	52389	SUR	COPD	Sputum	S	S	S	R	R	R	S	-	S	-	ESBL	-
20	Parthasarathy	54	M	56080	SUR	DFS	Pus	S	S	S	S	S	S	S	-	S	-	-	-
21	Kalaimani	55	M	41433	CT	Hydropneumotho	Pus	S	R	S	S	S	S	S	-	S	-	-	-

S. No	Name	Age	Sex	IP.No	Ward	Diagnosis	Sample	AMK	GM	CIP	CAZ	Cefepime	PT	IMP	IMP MIC	MRP	Colistin	Resistance pattern	*bla* $_{NDM-1}$ */bla* $_{VIM}$
22	Annadurai	64	M	44821	RTU	Post renal	Drain	S	S	R	R	S	S	S	-	S	-	ESBL	-
23	Anandhan	47	M	56067	VS	R AK Amputati	Pus	S	S	S	S	S	S	S	-	S	-	-	-
24	Rajesh	45	M	53852	Ortho	Compoun d open #	Pus	S	S	R	R	S	S	S	-	S	-	-	-
25	Siva	84	M	46003	CT	L Hydropn	Pus	S	S	S	R	S	S	S	-	S	-	-	-
26	Suryabalan	19	M	45307	Neuro	RTA	Urine	R	R	R	R	R	R	S	-	R	S	MDR/MRIS	-
27	Janaki	42	F	57750	ENT	Surgical site	Pus	S	S	R	R	S	S	S	-	S	-	-	-
28	Manikandan	19	M	43700	SUR	Cellulitis	Pus	R	R	R	R	R	S	S	-	S	-	MDR/AmpC	-
29	Vijendran	38	M	59071	IMCU	CKD/Lup us	Endotrac heal	S	R	R	R	R	R	S	-	R	S	MDR/MRIS	-
30	Krishnamoorthy	45	M	54200	Neuro	RTA/hea d injury	Endotrac heal	S	S	S	R	R	R	S	-	S	-	MDR/AmpC	-
31	Sundaramoorthy	69	M	41178	SUR	Post operative	Sputum	S	S	S	S	S	S	S	-	S	-	-	-
32	Kowsalya	25	F	57511	IMCU	LRI	Sputum	S	S	S	S	S	S	S	-	S	-	-	-
33	Radhi	47	F	56914	TM	Loculated effusion	Bronchial wash	S	R	S	S	S	S	S	-	S	-	-	-
34	Sundaram	66	M	58674	Uro	UTI	Urine	S	R	R	R	R	R	S	-	R	S	MDR/MRIS	-
35	Janaki	42	F	57750	SUR	Non-healing	Pus	S	S	S	R	S	S	S	-	S	-	ESBL	-
36	Manikandan	18	M	43700	Ortho	BB Fracture	Pus	R	R	R	R	R	S	S	-	S	-	MDR/ESBL	-
37	Sathish	33	M	34903	Ortho	Crush injury	Pus	R	R	R	R	R	S	S	-	S	-	MDR/ESBL	-
38	Srinivasan	34	M	54770	Neuro	R Hemipleg	Sputum	S	S	S	R	R	S	S	-	S	-	ESBL	-
39	Natarajan	53	M	54809	Ortho	LRI	Sputum	S	S	S	S	S	S	S	-	S	-	-	-
40	Karthikeyan	57	M	52276	TM	Bronchie ctasis	Bronchial wash	S	S	S	S	S	S	S	-	S	-	-	-
41	Sampath	61	M	51273	MED	LRI	Sputum	S	S	S	R	R	R	S	-	S	-	AmpC	-
42	Ganesan	63	M	51375	SUR	Old PTB	Sputum	S	S	S	R	R	R	S	-	S	-	AmpC	-
43	Prakasam	85	M	57278	TM	CA R Lung	Bronchial wash	S	S	R	R	R	R	S	-	S	-	MDR/AmpC	-

S. No	Name	Age	Sex	IP.No	Ward	Diagnosis	Sample	AMK	GM	CIP	CAZ	Cefepime	PT	IMP	IMP MIC	MRP	Colistin	Resistance pattern	*bla* $_{NDM-1}$ */bla* $_{VIM}$
44	Revathi	55	F	55864	SUR	Cellulitis	Pus	S	R	S	S	S	S	S	-	S	-	-	-
45	Srinivasan	46	M	52113	Ortho	Crush injury	Pus	S	S	S	S	S	S	S	-	S	-	-	-
46	Papammal	65	F	56866	SUR	Cellulitis	Pus	S	S	R	R	S	S	S	-	S	-	-	-
47	Sudhakar	43	M	49245	SUR	CA Buccal mucosa	Pus	S	S	S	S	S	S	S	-	S	-	-	-
48	Adhilakshmi	56	F	57561	SUR	DFS	Pus	R	R	R	R	R	S	S	-	S	-	MDR	-
49	Anbarasan	29	M	52579	Neuro	Paraplegia	Catheter tip	S	S	S	R	S	S	S	-	S	-	ESBL	-
50	Siva kumar	38	M	58785	IMCU	COPD	Sputum	S	S	R	R	R	R	S	-	S	-	MDR/AmpC	-
51	Rajaram	70	M	59122	IMCU	VAP	Endotracheal	S	S	S	R	R	R	R	R	R	S	MDR/MBL	bla_{VIM}
52	Munusamy	35	M	58162	Ortho	Loculated effusion	Pleural fluid	R	R	R	R	R	R	R	R	R	S	MDR/MBL	bla_{NDM-1}
53	Nandhakumar	62	M	55777	MED	Pyothorax	Pus	S	R	S	S	S	S	S	-	S	-	-	-
54	Chandran	50	M	58893	MED	old PTB	Sputum	S	R	S	S	S	S	S	-	S	-	-	-
55	Indra	47	F	58414	MED	LRI	Sputum	S	S	S	R	R	R	S	-	S	-	ESBL	-
56	Sowmya	19	F	59175	MED	Bronchiectasis	Sputum	S	S	S	R	R	R	S	-	S	-	AmpC	-
57	Krishnapillai	75	M	59563	Uro	BPH	Urine	S	S	R	R	S	S	S	-	S	-	ESBL	-
58	Kalyani	60	F	51273	ENT	CSOM	Aural swab	S	S	S	S	S	S	S	-	S	-	-	-
59	Pachaiyammal	50	F	57856	IMCU	ARI	Endotracheal	S	R	R	R	R	R	R	I	R	S	MDR/MBL	Negative
60	Malarvizhi	61	F	51275	SUR	Wound infection	Pus	S	S	S	S	S	S	S	-	S	-	-	-
61	Jayalakshmi	51	F	48669	MED	Cellulitis	Pus	S	S	R	R	S	S	S	-	S	-	-	-
62	Rajkumar	37	M	58197	SUR	DFS	Pus	S	R	S	R	R	S	S	-	S	-	ESBL	-
63	Karthik	24	M	54445	MED	Pyothorax	Pus	S	S	S	S	S	S	S	-	S	-	-	-
64	Nythirkani	40	F	51059	SUR	Cellulitis	Pus	S	S	S	S	S	S	S	-	S	-	-	-
65	Saravanan	25	M	48518	Ortho	# Femur	Pus	S	R	R	S	S	S	S	-	S	-	-	-

S. No	Name	Age	Sex	IP.No	Ward	Diagnosis	Sample	AMK	GM	CIP	CAZ	Cefepime	PT	IMP	IMP MIC	MRP	Colistin	Resistance pattern	*bla* $_{NDM-1}$ */bla* $_{VIM}$
66	Kavitha	18	F	43769	MED	LRI	Sputum	S	S	S	R	S	S	S	-	S	-	-	-
67	Sampath	70	M	42759	ENT	Stridor	Pus	S	S	S	S	S	S	S	-	S	-	-	-
68	Mahendran	26	M	51066	Ortho	# BB forearm	Pus	R	R	R	R	R	S	S	-	S	-	MDR/ESBL	-
69	Venkatakrishna	27	M	52381	Nephro	CKD	Catheter tip	S	S	S	S	S	S	S	-	S	-	-	-
70	Annadurai	41	M	52600	TM	Bronchiectasis	Sputum	S	S	S	R	R	R	S	-	S	-	MDR/AmpC	-
71	Ekambaram	65	M	44162	IMCU	VAP	Endotracheal	R	R	S	R	R	R	S	-	S		MDR	-
72	Sundari	40	F	56107	Nephro	CKD	PD Fluid	S	S	R	R	S	S	S	-	S	-	-	-
73	Ranjith	45	M	51609	ENT	CSOM	Aural swab	R	R	R	R	R	R	S	-	S	-	MDR/AmpC	-
74	Karthik	32	M	52133	VS	Trauma	Pus	S	S	R	R	R	S	S	-	S	-	ESBL	-
75	Philomina	60	F	51072	TM	old PTB	Sputum	S	S	S	R	R	R	S	-	S	-	AmpC	-

MASTER CHART - ACINETOBACTER BAUMANNII

S. NO	Name	Age	Sex	IP.No	Ward	Diagnosis	Sample	AMK	GM	CIP	CAZ	PT	COT	TET	IMP	IMP MIC	MRP	Colistin	Resistance pattern	PCR for OXA-23
1	Jayaraman	80	M	51230	GICU	UTI	Urine	S	R	R	R	S	R	R	S	-	S	-	MDR/ESBL	-
2	Veeramani	42	M	54778	VS	Skin Graft	Pus	R	R	R	R	S	R	S	S	-	S	-	MDR/ESBL	-
3	Lakshmi	56	F	51287	MED	UTI	Urine	S	R	R	S	S	S	R	S	-	S	-	-	-
4	Umapathy	21	M	57156	Uro	UTI	Urine	S	S	S	R	S	S	R	S	-	S	-	ESBL	-
5	Mahamathi	58	M	56345	Uro	UTI	Urine	S	S	R	S	S	R	S	S	-	S	-	-	-
6	Venkatesan	45	M	48919	SUR	DFS	Pus	S	R	R	R	R	R	R	S	-	S	-	MDR/AmpC	-
7	Sivakumar	65	M	54215	SUR	Carbuncle	Pus	R	R	S	S	S	R	S	S	-	S	-	-	-
8	Shanmugam	55	M	53496	VS	Cellulitis	Pus	S	R	S	R	S	R	S	S	-	S	-	ESBL	-
9	Elammal	60	F	36945	NS	RTA/head injury	Endotracheal	S	S	S	R	S	R	R	S	-	S	-	ESBL	-
10	Janarthanan	80	M	53908	MED	Sepsis	Blood	S	R	S	R	R	R	R	R	I	R	S	MDR/MBL	Negative
11	Rekha	30	F	54375	SUR	RTA	Pus	S	R	R	R	R	R	S	S	-	S	-	MDR/AmpC	-
12	Selvam	65	M	47882	GICU	Sepsis	Blood	S	S	S	R	S	R	R	S	-	S	-	-	-
13	Kuruvammal	33	F	52114	Uro	UTI	Urine	S	R	R	R	R	S	R	S	-	S	-	MDR/AmpC	-
14	Sathish Kumar	29	M	50218	Ortho	# Femur	Pus	R	R	R	R	R	R	R	S	-	S	-	MDR/AmpC	-
15	Sathish	31	M	52689	Uro	Pyelonephritis	Urine	S	R	R	S	S	S	R	S	-	S	-	-	-
16	Ponni	73	F	56284	MED	UTI	Urine	S	R	R	R	S	R	S	S	-	S	-	MDR/ESBL	-
17	Dinesh Kumar	23	M	50533	Ortho	RTA	Pus	S	R	R	R	S	R	R	S	-	S	-	MDR	-
18	Padmavathy	52	F	58243	CT	SSI	Pus	R	S	S	R	S	R	S	S	-	S	-	ESBL	-
19	Ramesh	55	M	40851	Uro	Pyonephritis	Urine	R	S	R	R	S	R	R	S	-	S	-	MDR	-
20	Kishore	18	M	33932	Ortho	# Left Tibia	Pus	S	S	S	R	S	R	R	S	-	S	-	ESBL	-
21	Geetha	19	F	55256	Ortho	# Humerus	Pus	S	R	S	S	S	S	S	S	-	S	-	-	-

S. NO	Name	Age	Sex	IP.No	Ward	Diagnosis	Sample	AMK	GM	CIP	CAZ	PT	COT	TET	IMP	IMP MIC	MRP	Colistin	Resistance pattern	PCR for OXA-23
22	Poovarasan	18	M	57505	IMCU	Urosepsis	Blood	R	R	R	R	R	R	R	R	R	R	S	MDR/CRAB	Positive
23	Angiah	43	M	56538	MED	UTI	Urine	S	R	S	R	S	R	S	S	-	S	-	ESBL	-
24	Gayathri	19	F	55744	SUR	UTI	Urine	S	S	R	R	S	R	R	S	-	S	-	ESBL	-
25	Anandha sekar	70	M	57820	Uro	BPH	Urine	S	R	S	R	S	R	S	S	-	S	-	-	-
26	Kumaresan	22	M	57941	MED	Bronchiectasis	Sputum	S	S	S	R	S	R	S	S	-	S	-	-	-
27	Surya	19	M	57935	SUR	SSI	Pus	S	R	R	R	R	R	R	S	-	S	-	MDR/AmpC	-
28	Suresh	32	M	54344	NS	Ganglioc apsular	Endotracheal	S	R	S	R	R	R	R	S	-	S	-	MDR/AmpC	-
29	Rajendran	58	M	56916	TM	Old PTB	Sputum	S	S	S	R	S	S	R	S	-	S	-	ESBL	-
30	Kalaivani	55	F	56639	MED	Bronchiectasis	Sputum	R	R	S	R	R	R	R	R	R	R	S	MDR/MBL	-
31	Dhanalakshmi	24	F	57928	MED	UTI	Urine	R	R	R	R	S	R	S	S	-	S	-	MDR/ESBL	-
32	Manikandan	27	M	53109	Nephro	CKD	Urine	S	R	S	S	S	R	S	S	-	S	-	-	-
33	Krishnan	62	M	58743	SUR	DFS	Pus	R	R	R	R	S	S	R	S	-	S	-	MDR/ESBL	-
34	Karthik	27	M	58796	SUR	Abdominal injury	Pus	S	R	S	S	S	S	S	S	-	S	-	-	-
35	Arumugam	67	F	59197	IMCU	VAP	Endotracheal	R	R	R	R	R	R	S	R	R	R	S	MDR/AmpC/ MBL	Negative
36	Prasad	48	M	57529	IMCU	VAP	Endotracheal	S	R	R	R	R	R	S	R	R	R	S	MDR/CRAB	Positive
37	Nagaraj	45	M	59476	TM	COPD	Sputum	S	S	S	R	S	R	R	S	-	S	-	ESBL	-
38	Jayachandran	30	M	55436	Nephro	CKD	PD Fluid	S	S	S	S	S	R	S	S	-	S	-	-	-
39	Natraj	32	M	53487	RTU	Post renal	Drain	S	R	S	R	S	R	R	S	-	S	-	ESBL	-
40	Soundarajan	60	M	52762	IMCU	VAP	Endotracheal	R	R	R	R	R	R	R	R	R	R	S	MDR/CRAB/ AmpC	Positive
41	Anjalai	48	F	51279	CT	MVR	Pus	S	S	R	R	S	R	R	S	-	S	-	-	-
42	Vel	26	M	50062	SUR	Scrotal abscess	Pus	R	R	R	R	S	R	R	S	-	S	-	MDR/ESBL	-
43	Kalyani	45	F	54113	SUR	RTA	Pus	R	R	R	R	S	R	S	S	-	S	-	MDR/ESBL	-

S. NO	Name	Age	Sex	IP.No	Ward	Diagnosis	Sample	AMK	GM	CIP	CAZ	PT	COT	TET	IMP	IMP MIC	MRP	Colistin	Resistance pattern	PCR for OXA-23
44	Mal Cruz	65	M	53104	SUR	Cellulitis	Pus	R	R	R	R	S	R	R	S	-	S	-	MDR/ESBL	-
45	Valarmathi	39	F	52869	Nephro	CKD	Catheter tip	S	R	S	R	S	R	S	S	-	S	-	-	-
46	Chinnapillai	70	M	45637	Uro	BPH	Urine	S	R	R	S	S	S	R	S	-	S	-	-	-
47	Kalyani	75	F	42739	TM	Pleural effusion	Sputum	S	R	S	R	S	R	R	S	-	S	-	-	-
48	Manikandan	33	M	41329	Hepatology	DCLD	Ascitic fluid	S	R	S	S	S	S	S	S	-	S	-	-	-
49	Selvam	54	M	55241	IMCU	VAP	Endotracheal	R	R	R	R	R	R	R	R	R	R	S	MDR/CRAB/ AmpC	Positive
50	Srinivasan	70	M	53621	MED	LRI	Sputum	S	S	S	R	R	R	S	S	-	S	-	ESBL/AmpC	-
51	Arumugam	45	M	51025	NS	RTA	Endotracheal	R	R	R	R	S	R	R	S	-	S	-	MDR/ESBL	-
52	Murugesan	50	M	53126	Nephro	CKD	PD Fluid	S	S	S	S	S	R	S	S	-	S	-	-	-
53	Sivasankar	47	M	43789	RTU	Post renal	Drain	R	R	S	R	R	R	R	S	-	S	-	MDR/ESBL/ AmpC	-
54	Narasiman	66	M	45762	IMCU	Sepsis	Blood	R	R	R	R	R	R	R	R	I	R	S	MDR/CRAB	Negative
55	Rafi	19	M	48743	NS	RTA/head injury	Endotracheal	S	R	R	R	R	R	S	S	-	S	-	MDR/AmpC	-
56	Dhanakarthi	35	M	56735	MED	UTI	Urine	S	R	R	R	R	R	S	S	-	S	-	MDR/AmpC	-
57	Elumalai	45	M	53478	MED	PTB	Sputum	S	S	S	R	S	R	S	S	-	S	-	ESBL	-
58	Vadivukarasi	18	F	56790	IMCU	AIHA	Blood	S	R	R	R	R	R	R	S	-	S	-	MDR/AmpC	-
59	Sophia	22	F	59459	ISCU	Appendicular	Blood	S	R	R	R	R	R	S	S	-	S	-	MDR	-
60	Vadivel	36	M	59466	CT	VAP	Endotracheal	R	S	R	R	R	R	R	S	-	S	-	MDR/AmpC	-
61	Philomina	60	F	52373	GICU	LRI	Sputum	S	S	S	S	S	R	S	S	-	S	-	ESBL	-
62	Kalyani	75	F	58760	MED	Pleural effusion	Sputum	S	S	S	R	S	S	S	S	-	S	-	-	-
63	Manikandan	33	M	58158	Hepatology	DCLD	Ascitic fluid	S	R	S	S	S	S	S	S	-	S	-	-	-
64	Kanagaraj	22	M	57389	Ortho	# BB leg	Pus	S	S	S	R	R	R	R	S	-	S	-	AmpC	-
65	Savithri	40	F	59226	CT	LA Myxoma	Urine	R	R	R	R	R	R	R	R	R	R	S	MDR/CRAB	Negative

S. NO	Name	Age	Sex	IP.No	Ward	Diagnosis	Sample	AMK	GM	CIP	CAZ	PT	COT	TET	IMP	IMP MIC	MRP	Colistin	Resistance pattern	PCR for OXA-23
66	Ranjith	45	M	56438	Ortho	# BB leg	Pus	R	R	R	R	S	R	S	S	-	S	-	MDR/ESBL	-
67	Vasantha	55	F	48762	MED	UTI	Urine	S	S	S	R	R	R	S	S	-	S	-	AmpC	-
68	Ekambaram	65	M	55487	NS	Head injury	Endotracheal	R	R	R	R	R	R	S	S	-	S	-	MDR/AmpC	-
69	Venkatakrishna	27	M	53286	MED	LRI	Sputum	S	R	S	S	S	R	S	S	-	S	-	-	-
70	Sundari	40	F	57890	Nephro	CKD	PD Fluid	S	R	S	R	S	R	S	S	-	S	-	ESBL	-
71	Saroja	65	F	49875	ISCU	SSI	Pus	S	R	R	R	S	S	S	S	-	S	-	MDR/ESBL	-
72	Narimani	68	F	56742	IMCU	Stridor/VAP	Endotracheal	R	R	R	R	R	R	R	R	R	R	S	MDR/CRAB	Negative
73	Swaminathan	68	M	54677	MED	LRI	Sputum	R	R	R	R	S	R	S	S	-	S	-	MDR/ESBL	-
74	Ramesh	37	M	53771	Nephro	Cellulitis	Pus	S	S	R	R	S	R	R	S	-	S	-	-	-
75	Tamilarasi	40	F	52651	NS	RTA	Urine	S	R	S	R	R	S	S	S	-	S	-	MDR/AmpC	-

LEGENDS FOR MASTER CHART

# BB	–	Fracture both bone
AMK	–	Amikacin
AmpC	–	AmpC Beta-lactamase
ARI	–	Acute Respiratory Illness
BPH	–	Benign Prostatic Hypertrophy
CAZ	–	Ceftazidime
CIP	–	Ciprofloxacin
COPD	–	Chronic Obstructive Pulmonary Disease
COT	–	Trimethoprim-sulfamethoxazole
CRAB	–	Carbapenem resistant *A.baumannii*
CSOM	–	Chronic Suppurative Otitis Media
CT	–	cardiothoracic unit
DFS	–	Diabetic Foot Syndrome
ESBL	–	Extended Spectrum Beta-lactamase
GICU	–	Geriatric Intensive care unit
GM	–	Gentamicin
I	–	Intermediate
IMCU	–	Intensive Medical care unit
IMP	–	Imipenem
ISCU	–	Intensive Surgical care unit
LRI	–	Lower Respiratory tract Infection
MBL	–	Metallo Beta-lactamase

MDR	–	Multidrug Resistant
MED	–	Medicine
MIC	–	Minimum Inhibitory Concentration
MRIS	–	Meropenem Resistant Imipenem Sensitive
MRP	–	Meropenem
NDM-1	–	New Delhi Metallo Beta-lactamase
Nephro	–	Nephrology
NS	–	Neurosurgery
Ortho	–	Orthopedics
OXA	–	Oxacillinase gene
PT	–	Piperacillin-tazobactam
PTB	–	Pulmonary Tuberculosis
R	–	Resistant
RTA	–	Road Traffic Accident
RTU	–	Renal Transplant Unit
S	–	Sensitive
SUR	–	Surgery
TET	–	Tetracycline
TM	–	Thoracic Medicine
Uro	–	Urology
UTI	–	Urinary Tract Infection
VAP	–	Ventilator Associated Pneumonia
VIM	–	Verona Integron mediated Imipenemase
VS	–	Vascular Surgery

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI 600 003

EC Reg.No.ECR/270/Inst./TN/2013
Telephone No.044 25305301
Fax: 011 25363970

CERTIFICATE OF APPROVAL

To
Dr.M.Sowndarya
Post Graduate in M.D. Microbiology
Madras Medical College
Chennai 600 003

Dear Dr.M.Sowndarya,

The Institutional Ethics Committee has considered your request and approved your study titled **"CHARACTERIZATION AND COLISTIN SUSCEPTIBILITY OF CARBAPENEM RESISTANT ISOLATES OF PSEUDOMONAS AERUGINOSA AND ACINETOBACTER BAUMANNII IN A TERTIARY CARE HOSPITAL" NO. 25042016.**

The following members of Ethics Committee were present in the meeting hold on **05.04.2016** conducted at Madras Medical College, Chennai 3

1.Dr.C.Rajendran, MD.,	:Chairperson
2.Dr.Isaac Christian Moses,MD.Ph.D.Dean(FAC)MMC,Ch-3	:Deputy Chairperson
3.Prof.Sudha Seshayyan,MD., Vice Principal,MMC,Ch-3	: Member Secretary
4.Prof.B.Vasanthi,MD., Prof.of Pharmacology.,MMC,Ch-3	: Member
5.Prof.P.Raghumani,MS, Prof. of Surgery,RGGGH,Ch-3	: Member
6. Prof.Md.Ali,MD.,DM.,HOD-MGE, MMC,Ch-3	: Member
7.Prof.Baby Vasumathi, Director, Inst. of O&G,Ch-8	: Member
8.Prof.K.Ramadevi,MD, Director,Inst.of Bio-Chem,MMC,Ch-3	: Member
9.Prof.M.Saraswathi,MD.,Director, Inst.of Path,MMC,Ch-3	: Member
10.Prof.Srinivasagalu,Director,Inst.of Int.Med.,MMC,Ch-3	: Member
11.Tmt.J.Rajalakshmi, JAO,MMC, Ch-3	: Lay Person
12.Thiru S.Govindasamy, BA.,BL,High Court,Chennai	: Lawyer
13.Tmt.Arnold Saulina, MA.,MSW.,	:Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

Member Secretary – Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE
CHENNAI-600 003